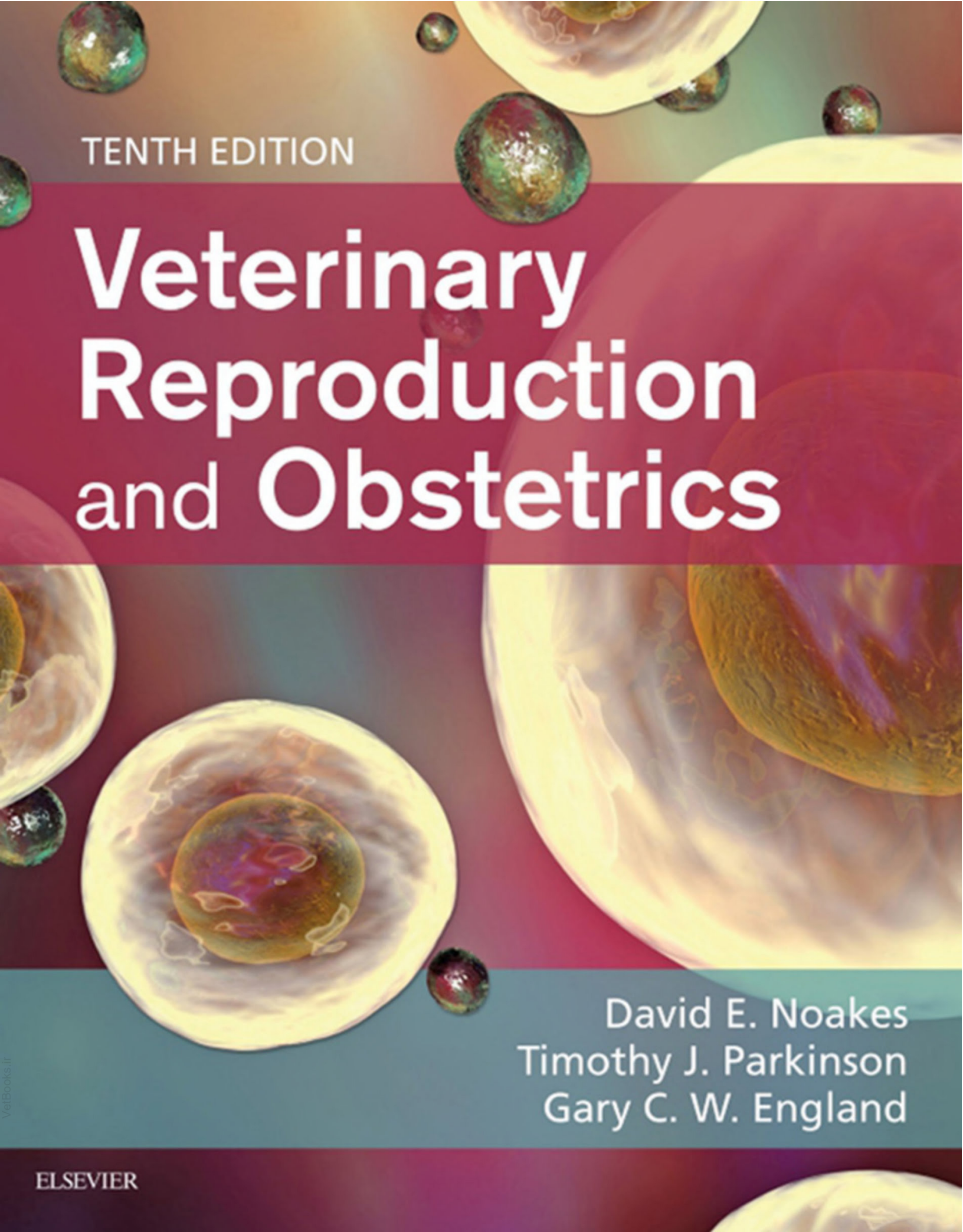
TENTH EDITION
Veterinary Reproduction and Obstetrics
David E. Noakes
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Veterinary Reproduction and Obstetrics

TENTH EDITION

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Veterinary Reproduction and Obstetrics

TENTH EDITION

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Preface

During the many hours spent revising the book to produce this new 10th edition, as editors/authors we have often thought how previous editions influenced our own professional activities and scientific interests.

The book was first published in the English Language in 1938, as a translation of the original German text by Professor Franz Benesch, who was Professor and Director of the Obstetrical Clinic of the Veterinary High School Vienna, entitled: 'Geburtshilfe bei Rind und Perd', which is literally 'Help with birth in cows and horses'. The English translation, first produced in 1938, was under the editorship of Professor John George Wright, who was Professor of Veterinary Surgery at the University of Liverpool, having previously been Professor of Surgery at the Royal Veterinary College. Both authors were great clinicians with outstanding teaching and practical skills. Thus their writings were based very closely on their personal experiences.

The first English translation of Benesch's original text in German published in 1938 was, as its title implies, almost entirely devoted to a text on dystocia and its treatment in cattle and horses. In the preface to the first joint author edition published in English 1951, it is apparent from comments in the preface that JG Wright, having sought the views and suggestions of others working in the discipline, included these in the new book. The subtitle to the book recognises this, stating: "—Including certain aspects of the physiology and pathology of reproduction in domestic animals". To some extent this underemphasises the scope of the book because in Part 1, for example it describes normal parturition in the sow, bitch and queen cat, whilst in Part 3 there are chapters on the caesarean operation in the bitch and queen cat as well as others on infertility in the mare, cow and bitch. Thus in 1950, when the book was being written, someone with the vision of JG Wright recognised that veterinarians of the future would need to diversify both their interests and expertise.

This change in emphasis and increased diversity has continued in previous editions, and is even greater reflected in this new 10th edition published nearly 80 years after Benesch's first book. Firstly, the diversity of species, these range from small mammals which are kept as pets and for biological research, domestic poultry and camelids, zebu cattle, buffalo and Asian Elephants for example, as well as what are considered to be more traditional domesticated species. Secondly, the wide nationality of the contributors; we have in the 10th edition recruited expert contributors from 14 different countries. Thirdly, whereas in the first edition there was no reference to the use of relatively simple reproductive technology such as artificial insemination, despite the report of the first successful use of the technique in the dog in 1780 and the successful transportation of semen overseas from sheep followed by AI in 1935, in this 10th edition a wide range of advanced reproductive biotechnologies are described and discussed.

As with previous editions, the book is intended primarily for veterinary, veterinary nursing, and animal science/agricultural students, as well as being a source of reference for anyone who has an interest in the biology of reproduction, particularly those species which have been subject to varying degrees of domestication.

We wish to thank all of the authors to the 10th edition for their hard work, and sharing their knowledge and expertise with us, and the subsequent readers of this new edition. It is a reflection of how our knowledge of reproductive biology has expanded since the sole author first edition published in 1938. We would also like to thank our families for their tolerance during the long gestation of the book, which fortunately did not quite become a prolonged gestation, requiring artificial induction.

Finally we would also like to thank Peter Parkinson for redrawing a substantial number of the figures, the staff at Elsevier for their expert help, particularly Ellen Wurm-Cutter in collecting the chapters from authors and latterly Carol O'Connell and Elizabeth Kilgore for the cheerful, professional and efficient way in which they have edited and proofed the chapters to produce a high quality textbook.

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PART 1

Basic Physiology

1 Reproductive Physiology of the Female, 2
Bob Robinson and David E. Noakes

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1

Reproductive Physiology of the Female

BOB ROBINSON AND DAVID E. NOAKES

In nature, generally, animals breed once annually, with parturition occurring in the spring at a time that is most favourable for the progeny. As a consequence, the early neonatal period of their life coincides with a period of increasing light and warmth and at the time when food for the mother is most abundant to ensure adequate lactation. The domestication of animals resulted in enhanced feeding and provision of housing such that the breeding season tends to be lengthened; in some species, e.g., cattle, they may breed at any time during the year. However, all domesticated animals show a tendency to revert to the natural breeding season, as evidenced by reduced fertility during summer and early autumn in sows.

For a female animal to reproduce, she must be mated and hence must attract the male and be sexually receptive (she is in oestrus or heat). All domestic mammals show recurring periods of sexual receptivity, or oestrous cycles, which are associated with the ripening in the ovaries of one or more Graafian (antral) follicles (Fig. 1.1), and culminate in the shedding of one oocyte from each ovulatory follicle. If a fertile mating occurs, then pregnancy may ensue.

The Oestrous Cycle and Its Phases

Traditionally, the oestrous cycle is divided into a number of phases.

Prooestrus

This is the phase immediately preceding oestrus. It is characterised by a marked increase in activity of the reproductive system. There are the final maturation stages of follicular growth, alongside regression of the corpus luteum (CL) from the previous cycle (in polycyclic species). The uterus enlarges very slightly, with the endometrium becoming congested and oedematous, and its glands show evidence of increased secretory activity. The vaginal mucosa becomes hyperaemic, the number of cell layers of the epithelium start to increase, and the superficial layers become cornified. The bitch shows external evidence of prooestrus with vulvar oedema, hyperaemia and a sanguineous vulvar discharge.

Oestrus

This is the period of acceptance of the male. The onset and end of the phase are the only accurately identifiable points in the oestrous cycle and hence are used as the reference points for determining cycle length. The animal usually seeks out the male and 'allows' him to mate her. The uterine, cervical and vaginal glands secrete increased amounts of mucus; the vaginal epithelium and endometrium become hyperaemic and congested, and the cervix is relaxed.

Ovulation occurs during this phase of the cycle in all domestic species with the exception of the cow, when it occurs about 12 hours after the end of oestrus. Ovulation is a spontaneous process in most domestic species, with the exception of the cat, rabbit and camelids in which it is induced by the act of coitus.

During prooestrus and oestrus, there is follicular growth in the absence of functional CLs; the main ovarian hormone produced is oestradiol. Prooestrus and oestrus are frequently referred to collectively as the follicular phase of the cycle.

Metoestrus

This is the phase succeeding oestrus. The granulosa and theca cells of the ovulated follicle give rise to lutein (luteal) cells, which are responsible for the formation of the CL. There is a reduction in the amount of mucus secretion from the uterine, cervical and vaginal glands.

Dioestrus

This is the period of the CL. The uterine glands undergo hyperplasia and hypertrophy, the cervix becomes constricted and the secretions of the genital tract are scant and sticky, while the vaginal mucosa becomes pale. The CL is fully functional during this phase and secretes large amounts of progesterone.

The period of the oestrous cycle when there is a functional CL is sometimes referred to as the luteal phase of the cycle to differentiate it from the follicular phase. Because in most of our domestic species oestrus is the only readily identifiable phase of the oestrous cycle, there is some merit in polyoestrous species to divide the cycle into oestrus and interoestrus, the latter including prooestrus, metoestrus and dioestrus. Another alternative division can be into follicular and luteal phases.

Anoestrus

This is the prolonged period of sexual rest, during which the genital system is mainly quiescent. Follicular development is minimal, and the CLs, although identifiable, have regressed and are nonfunctional. Secretions are scanty and tenacious, the cervix is constricted and the vaginal mucosa is pale.

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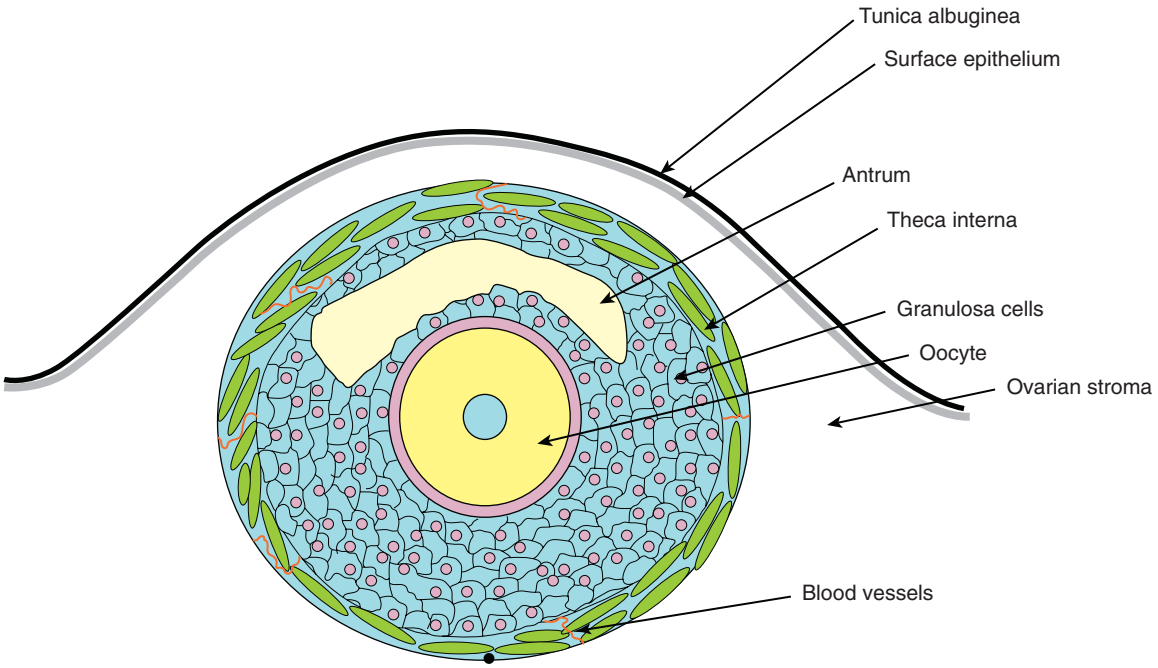

• **Fig. 1.1** Crosssection of a graafian follicle. (Drawn by Dr Mhairi Laird.)

Natural Regulation of Cyclical Activity

Regulation of cyclical activity in the female is a complex process. With the development of techniques, particularly those involving hormone assays and the application of new molecular biological techniques, there is a continual advance in the knowledge and understanding of the mechanisms involved. Although much of the early work was done on laboratory animals – notably the rat and guinea pig – there is now much more information about domestic species, although there are still areas, particularly in the bitch, that are not fully understood.

The central control of cyclical activity is the hypothalamic–pituitary–ovarian axis. At one end of this axis, there is the influence of the extrahypothalamic areas – the cerebral cortex, thalamus and midbrain – and their role in coordinating stimuli such as light, olfaction and touch. At the other end is the influence of the uterus upon the ovary.

Seasonal

The pineal gland plays a critical role in controlling reproductive activity in seasonal breeders by regulating the release of follicle stimulating hormone (FSH), luteinising hormone (LH) and prolactin. Much of the focus has been on the action of melatonin on the hypothalamus and pituitary gland. Melatonin is produced by the pineal gland during periods of darkness, with increasing melatonin secretion driving the reproductive response in 'short-day' breeders such as goats and sheep (Lincoln & Hazlerigg 2010). In 'long-day' breeders such as the horse, the increasing daylight length will 'switch-on' the hypothalamus–pituitary–ovarian–axis. In this case it is decreasing melatonin secretion that drives the stimulation. The integral role that season plays in controlling reproduction in seasonal breeders is extensively discussed in Chapter 3.

Hypothalamic and Anterior Pituitary Hormones

The hypothalamus is responsible for the control of the release of gonadotrophins (FSH and LH) from the anterior pituitary by the action of specific releasing and inhibitory substances. These are secreted by the hypothalamic neurons and are carried from the median eminence of the hypothalamus by the hypothalamic–hypophyseal portal system. In 1971 the molecular structure of porcine gonadotrophin (GnRH) was determined as being a decapeptide (Matsuo et al. 1971) and subsequently synthesised (Geiger et al. 1971). Over the past decade, a newly discovered neuropeptide called kisspeptin has been shown to act as a 'gatekeeper' for GnRH release. Kisspeptin is secreted from hypothalamic neurons and stimulates GnRH by acting directly on GnRH neurons (Clarke & Arbabi 2016). Kisspeptin is an important regulator of the timing of puberty, as well as the regulation of gonadotrophin secretion in seasonal breeders, and is discussed in Chapter 3.

It is clear that both gonadotrophins (FSH and LH) are located within the same gonadotrophic cell, and the exogenous administration of GnRH stimulates the release of both FSH and LH (Lamming et al. 1979). However, there is strong evidence that there is differential secretion of FSH and LH from the anterior pituitary gland and that it changes in the different stages of the oestrous cycle. It appears that GnRH is critical for the basal and pulsatile secretion of LH, which is particularly evident during the preovulatory LH surge. In contrast, the secretion of FSH secretion is more independent from direct GnRH receptor signalling (Pawson & McNeilly 2005).

There is good evidence that in domestic species the secretion of FSH and LH is controlled by two functionally separate, but superimposable, systems with two hypothalamic centres involved in controlling these two systems (Fig. 1.2). These are: (1) the episodic/tonic system, which is responsible for the continuous basal secretion of gonadotrophin that stimulates the growth of

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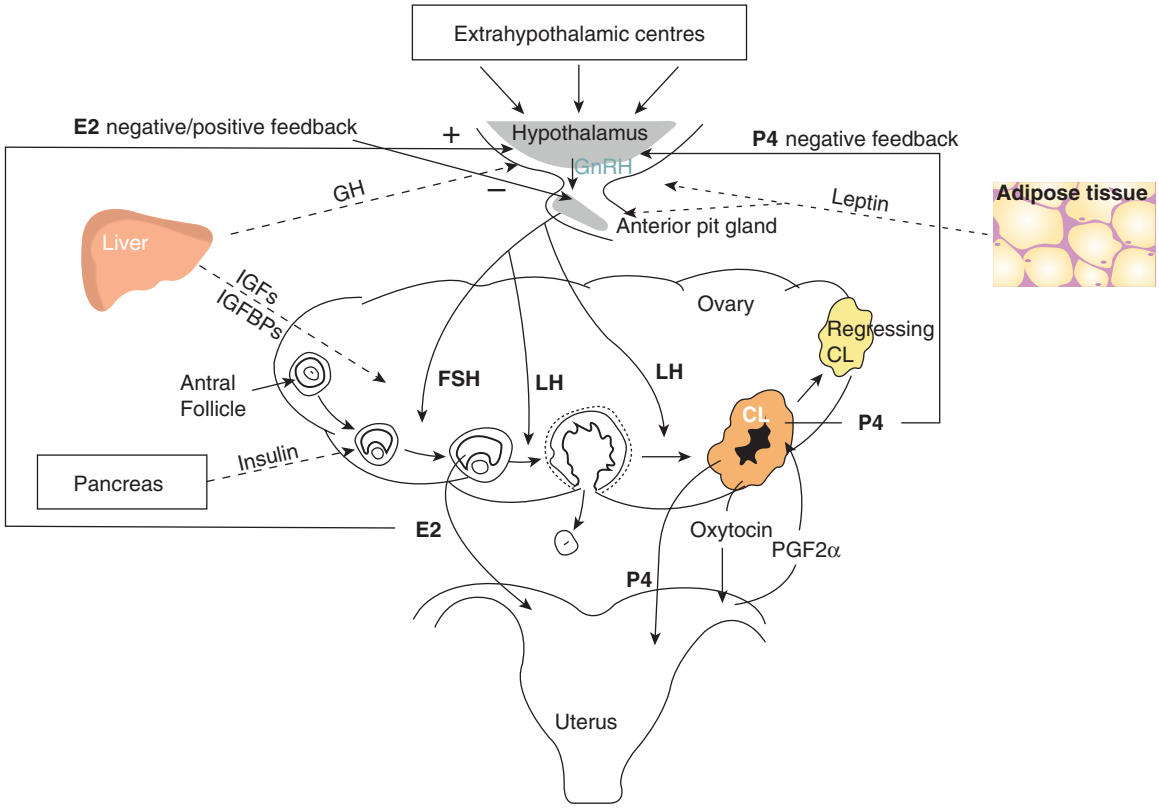

• **Fig. 1.2** Endocrine control of cyclical reproductive activity. *Solid line,* HPO axis; *dashed line,* extra HPO axis inputs; *GH,* growth hormone; *IGFs,* insulin-like growth factors; *IGFBPs,* insulin-like growth factor binding proteins; PGF_{2alpha}, prostaglandin F_{2alpha}. (Adapted from Lamming et al. 1979.)

both germinal and endocrine components of the ovary; this predominates during the luteal phase when LH pulses are described as high amplitude, low frequency (every 4-8 hours); and (2) the surge system, which controls the short-lived massive secretion of gonadotrophin, particularly LH, and is responsible for ovulation. This is evident at the end of the follicular phase, when high amplitude LH pulses occur more frequently (every 1-2 hours) resulting in a LH surge that is at least tenfold higher than a tonic pulse of LH (Rahe et al. 1980). It is important that this occurs in most domestic mammals as they are spontaneous ovulators. Notable exceptions are the cat, rabbit and camelids, in which ovulation is induced by the stimulation of sensory receptors in the vagina and cervix at coitus. This initiates a neuroendocrine reflex which ultimately results in the activation of GnRH neurons in the surge centre and release of a surge of LH.

There is a precise interplay between the ovary and the hypothalamus–pituitary, as the anterior pituitary not only has a direct effect on ovarian function (e.g., stimulating folliculogenesis, follicular maturation, ovulation and CL formation), but the ovary also has an effect upon the hypothalamus and anterior pituitary. This is principally mediated by oestradiol, produced by the maturing follicle, and by progesterone, produced by the CL.

The episodic/tonic hypothalamic release centre is influenced by the negative feedback effect of oestradiol (below threshold levels) and progesterone. Indeed, progesterone has a profound negative effect on the tonic and surge centres, which appears to be particularly important in ruminants (Lamming et al. 1979). In the cow, ewe and sow (and probably in other domestic species) FSH secretion is additionally controlled by a number of ovarian-derived peptide hormones. The first characterised was inhibin, which is produced by the granulosa cells of large antral follicles and accumulates in the follicular fluid (Fig. 1.1); it is also produced in the testis by Sertoli cells (see Chapter 2). Inhibin and oestradiol act in concert to suppress FSH secretion. Inhibin, which is produced by all antral follicles, has a longer half-life and sets the overall level of negative feedback, whereas oestradiol, which is produced only by those antral follicles that have the potential for ovulation, is responsible for the day-to-day fluctuations (Baird et al. 1991). Two other peptide hormones that have been isolated from ovarian follicular fluid are designated: activin, which stimulates, and follistatin, which suppresses FSH secretion. Over recent years, it has become increasingly apparent that both activin and inhibin have intraovarian functions such as follicle growth and modulating steroid production (Knight et al. 2012).

The positive feedback effect of oestradiol on hypothalamic–pituitary function is well demonstrated in farm animals because the preovulatory surge of oestradiol stimulates the release of LH, which is absolutely critical for the process of ovulation and CL formation. The response of the anterior pituitary to GnRH is influenced by the levels of ovarian steroids so that there is increased responsiveness shortly after the level of progesterone declines and that of oestradiol rises (Lamming et al. 1979). There are probably self-regulatory mechanisms controlling gonadotrophin secretion acting locally within the anterior pituitary and hypothalamus.

Tonic release of gonadotrophins, especially LH, does not occur at a steady rate but in a pulsatile fashion in response to a similar release of GnRH from the hypothalamus. The negative feedback of progesterone is mediated via a reduction in pulse frequency of gonadotrophin release, whereas oestradiol exerts its effect by reducing pulse amplitude. The onset of cyclical activity after parturition,

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at puberty or at the start of the breeding season is associated with increased pulse frequency of tonic gonadotrophin secretion. For example, when the ram is in contact with ewes before the start of the breeding season, there is increased frequency of pulsatile LH release, which stimulates the onset of ovarian cyclical activity (Karsch et al. 1984).

Folliculogenesis

The current evidence indicates the formation of follicles is completed during fetal life, such that at birth, there are approximately a half to 1 million follicles. Each follicle will contain an oocyte that is arrested in meiosis I. The majority of these follicles will remain at the primordial stages until puberty when, in cohorts, they will undergo a 'committed' transition into primary follicles (Scaramuzzi et al. 2011). The exact regulation of this process remains unclear but it is likely to involve activation by Kit ligand and mTORC1 (mammalian target of rapamycin complex 1). Conversely, anti-Müllerian hormone (AMH), PTEN (phosphatase and tensin homologue) and forkhead box transcription factors are inhibitory. The protein complex mTORC1 might play an integral role because it is activated by various cues such as nutrients, energy, stress and oxygen (Monniaux 2016). Simultaneously, the oocyte will increase in size and start to form its protective glycoprotein coat called the zona pellucida. The primary follicle cohort will then progress into preantral (secondary) follicles once it has multiple granulosa cell layers alongside the recruitment of the vascularised theca layer. These preantral follicles grow in size, and at 0.1 to 0.5 mm will form a fluid-filled antrum and become antral follicles. Antral follicles are also known as tertiary or Graafian follicles. These early stages of folliculogenesis occur in the absence of gonadotrophin support and are likely controlled by intraovarian growth factors such as insulin-like growth factors (IGF), members of the transforming growth factor beta (TGFB) superfamily, and vascular endothelial growth factor (VEGF) (Hunter et al. 2004, Scaramuzzi et al. 2011). However, it is likely that the development is stimulated by FSH. An emergent concept is that the oocyte plays an active role in regulating folliculogenesis and that there is extensive interplay between the oocyte, granulosa and theca cells. It is widely recognised that the oocyte secretes two important growth factors: growth differentiation factor 9 (GDF9) and bone morphogenetic protein 15 (BMP15). Naturally occurring point mutations in these genes and their receptors (e.g., BMP receptor type 1B) are known to affect ovulation rate in sheep. For example, Booroola ewes will typically yield 4 to 5 offspring as a consequence of an enhanced ovulation rate. In these sheep, the point mutation, known as $FecB^{B}$, was characterised to affect the BMPR1B gene (McNatty et al. 2005). Importantly, the whole process from primordial follicle to ovulation takes 4 to 6 months in ruminants but is slightly shorter in pigs (Hunter et al. 2004), and there is a loss of follicles, and consequently the oocyte, as result of atresia at all stages of folliculogenesis.

The important stage of folliculogenesis from a veterinary perspective is the continuous growth, development and regression (atresia) of antral follicles that occurs throughout the oestrous cycle, during pregnancy and in other reproductive stages. There appear to be two different patterns of follicular growth (Fortune 1994, Scaramuzzi et al. 2011). First, well-organised, wave-like patterns of follicular development occur throughout the oestrous cycle in horses, cattle, sheep, goats and buffalo or, in the case of camelids, during the periods of reproductive activity. Thus there are antral follicles present throughout the oestrous cycle, including the luteal phase, and these follicles are often similar in size to those that are preovulatory. However, in the sow there is no evidence of a wave-like pattern, but there is the presence of 30 to 50 intermediate-sized follicles (2-7 mm in diameter) during the luteal phase. From these, between 15 and 30 will grow and ultimately ovulate once the CL regresses and the follicular phase is initiated (approximately days 14 to 16 of the oestrous cycle). One explanation for such a system of folliculogenesis may be the large number of follicles that ovulate over a very short space of time in this species. The patterns of follicular development in individual species will be described separately below. The following terminology describing folliculogenesis is now generally accepted (Webb et al. 1999):

- **Recruitment**: gonadotrophin stimulation of a cohort of rapidly growing antral follicles
- **Selection**: the process whereby one or more of the recruited follicles are selected to develop further
- **Dominance**: the mechanism whereby one (the dominant follicle) or several codominant follicles undergo further maturation in an environment in which the growth and development of other antral follicles is suppressed

The pattern of follicular dynamics has been expertly described, particularly in ruminant species, by Adams (1999) and is briefly summarised here: The periodic increases in peripheral FSH concentrations are associated with the recruitment of small antral follicles and the emergence of the follicular wave. These follicles will continue to develop under the influence of FSH. This is followed by the selection of a dominant follicle, as the follicle becomes less dependent on FSH and acquires enhanced LH responsiveness. This is associated with the appearance of the LH receptor in the granulosa cells. The dominant follicle secretes inhibin and oestradiol that feedbacks on the hypothalamus–pituitary axis to reduce circulatory FSH concentrations. Thus any antral follicle that is still FSH-dependent will regress and undergo atresia. The dominant follicle will further develop and can remain dominant for several days. It will undergo its final maturation and ovulate if progesterone concentrations decline. However, if progesterone concentrations are maintained, it will regress as progesterone is suppressive to LH secretion, enabling FSH concentrations to increase and the recruitment of a new follicular wave. These periodic anovulatory follicular waves will continue to occur until there is an LH surge. Typically, within a given species there are an increased number of follicular waves when the oestrous cycle is longer. In all species the follicular dominance is enhanced during the first and last follicular wave of the oestrous cycle. The exact mechanism or mechanisms by which follicle becomes dominant and deviates from the subordinate follicles remain a mystery. Originally, it was hypothesised that the follicle with the largest diameter during selection was the follicle that became dominant. The frequent sequential ultrasonographic tracking of individual follicles during the selection and dominance phases has demonstrated that this hypothesis is an over-simplification (Mihm & Evans 2008, Ginther 2016). It is clear that the vascular bed is greater in dominant follicle enabling enhanced gonadotrophin and nutrient uptake. Based on this, Zeleznik et al. (1981) proposed that the degree of vascularisation within the follicle plays a critical role in the selection of the dominant follicle. The use of improved Doppler blood flow technologies has indicated that there is enhanced blood flow to the follicle that becomes dominant prior to deviation in the mare (Acosta et al. 2004) and cow (Ginther et al. 2017). It is widely recognised that the acquisition of LH receptors in the granulosa cell layer is critical for a follicle to achieve dominance. Recent transcriptomic approaches have yielded further insights (Evans et al. 2004, Mihm & Evans 2008, Liu et al.

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2009, Hatzirodos et al. 2014). These include the upregulation of transforming growth factor β signalling (e.g., inhibin A, BMP receptors), immune/inflammation signalling, and transcription factor as well as intercellular matrix adhesion.

Even during times such as pregnancy, anoestrus and postpartum, there is evidence of follicular growth and regression. Follicular waves have been identified in pregnant cows, ewes, doe goats, llamas and camels as well as during the puerperium, before the resumption of ovarian cyclical activity. However, the follicles tend to have a smaller diameter than those present in follicular waves of nonpregnant individuals (Evans 2003).

Ovarian Steroids

Progesterone plays a critically important role in the inhibition of the tonic mode of LH secretion (Goodman & Karsch 1980) by reducing LH pulse frequency. Progesterone is a major regulatory hormone that controls the oestrous cycle of domestic mammals. Thus when the circulatory concentrations of progesterone fall (e.g., during luteolysis), there is increased release of LH from the anterior pituitary. The subsequent increased frequency of LH pulses triggers the secretion of oestradiol, and its rapid rise stimulates the hypothalamic surge centre. Consequently, there is a surge of LH that will induce the final stages of follicular maturation, ovulation and the release of oocyte (McNatty et al. 1981).

In some species, notably the cow (see Fig. 1.29), there is also a concomitant surge in FSH, although its significance is unclear; it may be part of the 'ovulation-inducing' hormone complex. For this reason, it is probably incorrect to assign a separate and specific physiological role for the two pituitary gonadotrophins. Although steroidogenesis can be initiated by both FSH and LH, it would appear that only FSH can induce early antral follicular growth, but when the granulosa cells have matured, they are able to respond to endogenous LH; then LH is principally responsible for triggering ovulation.

Ovulation

The preovulatory LH surge triggers a series of cellular and molecular changes in the follicle that culminate in ovulation. Ovulation is a complex process**,** involving the measured destruction of the follicle enabling the release of the oocyte and conversion of the follicle into the CL. The three key events involved in ovulation are: (1) elevated local blood flow (hyperaemia) and increased vascular permeability stimulated by prostaglandin E_2 and VEGF; (2) the breakdown of the connective tissue within the tunica albuginea and the basement membrane of the ovulatory follicle. The activity of proteolytic enzymes (e.g., collagenase, plasmin) is triggered by a local rise in progesterone and PGE_2 production from the theca cells. Collectively, this will increase follicular fluid volume and weaken the apex of the follicle, leading it to protrude and form a stigma; and (3) the contractions of the smooth muscle within the theca externa layer of the follicle. These are stimulated by $PGF_{2\alpha}$ and may increase pressure locally to force the stigma to protrude more and eventually open up to release the oocyte and follicular fluid (Murdoch et al. 2010).

Formation of the Corpus Luteum

The CL is rapidly formed from the Graafian follicle after ovulation, primarily from the luteinisation of the granulosa and thecal cells; this is accompanied with intense angiogenesis. Collectively, this enables the CL to increase its mass to twentyfold over a period of 10 to 12 days (Reynolds & Redmer 1999; Mann et al. 2007). For some time it was assumed that once formed, it remained a relatively static structure. However, it is now known that when it is functionally mature, there is still rapid cell turnover, although there is little change in its size. The fully formed CL consists of a number of different cell types: the steroid-secreting large and small luteal cells, fibroblasts, smooth muscle cells, pericytes and endothelial cells (Woad & Robinson 2016). It has the greatest blood supply per unit tissue of any organ (Reynolds & Redmer 1999). In the ewe, based on volume, the large luteal cells comprise 25% to 35%, the small luteal cells 12% to 18% and vascular elements 11% (Rodgers et al. 1984). Although the CL develops as a result of ovulation, in some species, notably the bitch, there are early signs of luteinisation of the follicle before it has ovulated (Concannon 2011). The stimulus for the formation and maintenance of the CL probably varies within species. The hormones that are most likely to be involved are prolactin and LH, with some evidence that they act in conjunction with each other. The treatment of ewes, cows and pigs with LH antiserum causes a dramatic decline in luteal weight and progesterone content, and therefore prolactin is unlikely to be essential for normal luteal function. However, prolactin plays a critical role in maintaining the CL in rodents and the dog (Niswender et al. 2000, Concannon 2011).

Regression (Luteolysis) of the Corpus Luteum

The presence of a functional CL, by virtue of its production of progesterone, inhibits the return to oestrus by exerting a negative feedback effect upon the hypothalamus. This is most obvious during pregnancy. In the normal, nonpregnant female, oestrus and ovulation occur at fairly regular intervals, and the main control of this cyclical activity would appear to be the CL. Although it has been known for over 80 years that in certain species the uterus influences ovarian function (Loeb 1923), the exact mechanisms are now only just being completely understood (see review by Weems et al. 2006).

It is recognised that, in many species, removal of part or all of the uterus will result in the life span of the CL being prolonged (Du Mesnil Du Buisson 1961, Rowson & Moor 1967). These species include cattle, horses, sheep, goats and pigs. However, in the human, dog and cat the normal life span of the CL is unaffected by the absence of the uterus. In the cow, ewe and goat the luteolytic action of the uterine horn is directed exclusively to the CL on the adjacent ovary (Ginther 1974, McCracken et al. 1999). Thus if the uterine horn adjacent to the ovary with a CL is surgically removed, then the CL will persist. Conversely, if the contralateral horn is removed, then the CL will regress at the normal time.

It was not until 1969 that the substance responsible for luteolysis was identified, when the duration of pseudopregnancy in the rat was shortened by the injection of prostaglandin $(PG)F_{2\alpha}$ (Pharriss & Wyngarden 1969). This same substance is known to have potent luteolytic activity in the ewe, doe goat, cow, sow and mare. Although it has been proved only in ruminants and the guinea pig that $PGF_{2\alpha}$ is the natural luteolysin, it is likely that it is also true for the other species listed.

It appears that the endometrial-derived $PGF_{2\alpha}$ is transported directly from the uterus to the ovary. In the ewe it has been shown that the most likely route for transport of the substance is the middle uterine vein because, when all other vascular structures between the ovary and uterus were severed, there was still normal regression of the CL (Baird & Land 1973).

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In the mare no local effect can be demonstrated because, if the ovary is transplanted outside the pelvic cavity, luteal regression still occurs (Ginther & First 1971). It is generally assumed that in this species the $PGF_{2\alpha}$ is transported throughout the systemic circulation. In the sow $PGF_{2\alpha}$ is transported locally, but not exclusively, to the adjacent ovary. It has been shown that, following surgical ablation of segments of the uterine horns and provided that at least the cranial quarter of the uterine horn is left, regression of the CLs occurs in both ovaries (Du Mesnil Du Buisson 1961). In the bitch the mechanisms by which the lifespan of the CL is controlled is not fully understood, but in this species even in the absence of pregnancy, there is always a prolonged luteal phase.

The mechanisms whereby $PGF_{2\alpha}$ is transported to the ovary have not been conclusively shown in all species, but it is fairly well evaluated in the ewe and cow. In the former species, it appears that the close proximity of the ovarian artery and utero–ovarian vein is important, particularly because at their points of approximation the walls of the two vessels are thinnest and there is no anastomosis (Coudert et al. 1974). This allows leakage of $PGF_{2\alpha}$ from the uterine vein into the ovarian artery and thus to the ovary, by a form of countercurrent exchange through the walls of the vessels. It has been suggested that the variation in the response to partial or total hysterectomy in different species is probably due to differences in the relationships between the vasculature of the uterus and ovaries (Ginther 1974).

$PGF_{2\alpha}$ is a derivative of the unsaturated linolenic and arachidonic acids. It derived its name because it was first isolated from fresh semen and was assumed to be produced in the prostate gland. It is synthesised in the endometrium of a number of species (Horton & Poyser 1976), and in ruminants pulsatile secretion of $PGF_{2\alpha}$ is increased at and around the time of luteal regression (Mann et al. 1999). For complete luteal regression to occur, 5 to 8 episodic release of $PGF_{2\alpha}$ pulses at intervals of approximately 6 hours is required (Arosh et al. 2016). There is a positive feedback loop present whereby oxytocin secreted by the CL stimulates $PGF_{2\alpha}$ secretion. Thus each episode of $PGF_{2\alpha}$ release is accompanied by an episode of oxytocin release. Furthermore, $PGF_{2\alpha}$ stimulates further secretion of oxytocin from the ovary.

Luteal regression has two important components: (1) functional regression, in which the secretion of progesterone declines; this component is rapid, resulting in sharp decline in peripheral progesterone concentration; and (2) structural regression, in which there is physical involution of the CL and regression of luteal tissue; this process takes longer than the functional regression. The primary targets of $PGF_{2\alpha}$ in the initiation of luteal regression are the large luteal cells, which possess prostaglandin F receptors on their cell surface. Initially, it is the large luteal cells that decrease in size, followed by the reduction in the small luteal cell size. Simultaneously, there is a disruption to the luteal vasculature and infiltration of immune cells such as macrophages alongside the production of various cytokines such as tumour necrosis factor (Pate et al. 2012). There is a wealth of evidence indicating that luteal endothelial cells play an integral role in luteolysis, with $PGF_{2\alpha}$ acting on these cells. It appears that there is a transient increase in luteal blood flow following $PGF_{2\alpha}$ administration, which is mediated by nitric oxide-induced vasodilation. However, as luteolysis progresses, there is a gradual decline in blood flow as a consequence of vasoconstriction induced by elevated endothelin and angiotensin II levels (Meidan et al. 1999, Miyamoto et al. 2009).

The sensitivity of the uterus to oxytocin is determined by the upregulation of endometrial oxytocin receptors. In ruminants just prior to the time of luteal regression, they rise approximately 500-fold (Lamming & Mann 1995). Their concentrations are determined by the effects of progesterone and oestradiol, with high concentrations of progesterone after ovulation downregulating oxytocin receptor expression. Then, in the nonpregnant animal, oxytocin receptor expression in the luminal epithelium is upregulated on day 12 (sheep) and 16 (cow) (Mann et al. 1999). Although exogenous oestradiol can cause premature induction of oxytocin receptors, resulting in premature luteolysis (Hixon & Flint 1987), the exact role of oestradiol and the oestrogen receptor in the upregulation of oxytocin receptor remains controversial. In nonruminant species much less is known about the mechanisms by which luteolysis is upregulated.

The CL becomes more sensitive to the luteolytic effect of $PGF_{2\alpha}$ as it matures. The early (growing) CL is unresponsive to $PGF_{2\alpha}$. The refractoriness of the CL to $PGF_{2\alpha}$ is likely to be due to the lack of attenuated molecular and cellular infrastructure rather than the absence of the prostaglandin FP receptor expression, which is abundant through the oestrous cycle (Miyamoto et al. 2009).

Leptin

Leptin has an important role in not only regulating food intake in man and domestic animals, but also in controlling reproduction and in particular the time of onset of puberty and fertility. Leptin is a 16-kDa protein (of 140 amino acids) that is synthesised by the white fat cells of adipose tissue and acts not only primarily on the hypothalamus, but also the anterior pituitary and reproductive tract because leptin receptors have also been identified in these structures (Dyer et al. 1997, Lin et al. 2000). The production of leptin is influenced by body condition/weight and thus informs the brain of the level of the body's energy stores (Clarke & Arbabi 2016). The interaction between food intake and the hypothalamic–pituitary axis has been shown by examining the effect of acute fasting and chronic feed restriction on both serum leptin and LH levels. In the cow and ewe there was a marked decrease in serum LH release coinciding with reduced leptin levels (Amstalden et al. 2000, Henry et al. 2001). However, in the pig an acute 24 hours fast caused a decrease in leptin, with no effect on LH (Barb et al. 2001). This indicates species differences, and emphasises the dangers of extrapolating from one to the other. The current evidence indicates that leptin is likely to exert its effects on LH secretion by modulating kisspeptin (Hausman et al. 2012).

Insulin-Like Growth Factor System

It is clear that the 'insulin-like growth factor (IGF) system' plays a fundamental role in the growth and selection of follicles, as well as luteal development in most of the domestic species (Webb et al. 2002, Mazerbourg et al. 2003). It is likely that the IGF system integrates the metabolic status of the animal with reproductive performance (Lucy 2008, Hernandez-Medrano et al. 2012, Wathes 2012). The system comprises a number of different, but related, elements. They are: (1) two ligands, IGF1 and IGF2; (2) type 1 and type 2 IGF receptors; and (3) six principal IGF-binding proteins (IGFBPs), which have high but varying affinities for binding both IGF1 and IGF2. The predominant source of IGF1 is from the circulation, with its production by the liver increased by growth hormone (GH), whereas IGF2 is more locally produced in the follicle and CL (Lucy 2008). The circulatory levels of IGF1 parallel the energy status of that animal in several species; for example, plasma IGF1 levels decline during the period of negative energy balance postpartum in dairy cows (Lucy 2008,

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Wathes 2012). The principal signalling receptor is the IGF type 1 receptor, which is abundantly expressed throughout the ovary and uterus. The IGFBPs are ubiquitous in all biological fluids, including follicular fluid; however, in blood the most abundant is IGFBP3. The bioavailability of IGF1 and IGF2 is reduced when they are bound to their binding proteins, and classically, it was believed that IGFBP inhibited the action of IGFs. However, there is good evidence that the IGFBPs can potentiate IGF activity by increasing their half-life and maintaining the local tissue IGF level (Mazerbourg et al. 2003). In addition, there is also a protease that degrades IGFBPs in the follicle, called pregnancy-associated plasma protein (PAPP)-A. The action of PAPP-A on IGFBPs is to release and enable IGF1 and IGF2 to be biologically active. How does the insulin-like growth factor system function? IGFs stimulate follicular growth by stimulating granulosa cell proliferation, as well as the emergence of a dominant follicle, by sensitising follicular granulosa cells to the effects of FSH and LH. In the latter stages of follicular development, IGF1 is more involved with follicular maturation and differentiation (Mazerbourg et al. 2003). Both IGF1 and IGF2 increase oestradiol production by the granulosa cells (Mazerbourg et al. 2003). The intrafollicular levels of several IGFBPs increase during follicular atresia, and they have been proposed as markers of atretic follicles. PAPP-A has been identified in bovine, equine, ovine and porcine preovulatory follicles, concomitant with decreased IGFBP levels in preovulatory follicles (Mazerbourg et al. 2003). In the CL, IGF1 is known to stimulate progesterone by upregulating key steroidogenic proteins and has been proposed to stimulate the intense neovascularisation after ovulation (Webb et al. 2002).

Prolactin

The exact role of prolactin in controlling reproduction in many domestic species is still largely speculative, and in many cases it is only possible to extrapolate from studies in the traditional laboratory species. Unlike other anterior pituitary hormones, which require hypothalamic stimulation, it appears that prolactin secretion from lactotrophic cells of the anterior pituitary gland is spontaneous. The predominant regulatory signal is inhibitory, which is mediated by the hypothalamus-derived neurotransmitter dopamine (Bouilly et al. 2012). There is some evidence to suggest that dopamine may have a dual role as a stimulant of prolactin secretion, rather like a prolactin-releasing factor. The best characterised role for prolactin is its activity on the CL, particularly in rodents and dogs. In these species, prolactin is essential for CL function and the inhibition of prolactin secretion leads to luteal regression (Concannon 2011).

Role of Opioids

There has been interest directed towards the role of certain endogenous opioid peptides such as *beta*-endorphin and *met*-enkephalin, which are involved in regulating food intake. These substances are found in high concentrations in hypothalamic–hypophyseal portal blood, and the administration of exogenous opioid peptides inhibits FSH and LH secretion while stimulating the secretion of prolactin. If an opiate antagonist such as naloxone is infused, there is an increase in gonadotrophin concentrations in the plasma and the frequency of episodic gonadotrophin secretion. It is feasible that opioid peptides are a potential mechanism by which nutritional restriction, possibly via neuropeptide Y, suppresses the GnRH pulse generator (Diskin et al. 2003). The effect of opioids appears to be influenced by the background steroid environment. For example, in ewes naloxone increased the mean plasma concentration of LH and the episodic frequency in a high progesterone environment. However, in ovariectomised ewes or those with oestradiol implants, naloxone had no effect (Brooks et al. 1986). It is possible that the negative feedback of progesterone on LH release may be mediated via opioids (Brooks et al. 1986).

Stress

There are numerous stressors that can impact on domestic animals such as poor body condition, adverse temperature and certain management procedure. All these events elicit a coordinated endocrine response that can suppress normal oestrous behaviour and cyclicity. Often the disruption is temporary and, when stress is eliminated, normal cyclical activity resumes (Dobson et al. 2012). The exact mechanism whereby stress influences reproduction is poorly understood, but stress has an impact on the hypothalamus by reducing GnRH secretion and on the anterior pituitary gland by decreasing FSH and LH secretion. It is most likely that stress mediates its effect, at least in part, by elevating plasma cortisol (or corticosterone) levels, which is known to suppress LH pulse frequency. Indeed, it has been shown that the imposition of experimental stress during the follicular phase leads to a delay or complete suppression of the LH surge (Dobson & Smith 1998).

Ovarian Reserve and Ageing

Female mammals are born with a variable, but finite, number of follicles and oocytes in their ovaries, and as the animal ages the number of these will gradually decline and are never replenished. Because the formation of follicles in the ovary is completed by the time of birth, it has been hypothesised that the maternal environment during gestation could impact on the offspring's reproductive capabilities. This hypothesis is supported experimentally, when the offspring of cows nutritionally restricted through the first trimester had lower antral follicle counts in adulthood (Mossa et al. 2013).

In humans, once this ovarian reserve is nearly exhausted, then the menopause will commence. Although this does not occur in domesticated mammals, there is evidence that fertility declines in cows with a low AFC (Mossa et al. 2012) and a reduced response to superovulation (Evans et al. 2012). The exact association between AFC and fertility remains to be established. It is likely that the AFC and anti-Müllerian hormone (AMH), which are produced by the granulosa cells of preantral and small antral follicles, reflect the level of ovarian reserve (Ireland et al. 2008). This has led to the prospect of measuring the plasma AMH level as an indicator of ovarian reserve and fertility (Mossa et al. 2017). This measurement is routinely performed in women undergoing artificial reproductive technology programmes and is an excellent indicator of ovarian ageing in women (Nelson et al. 2012). There is minimal variation in the plasma AMH level throughout the oestrous cycle; thus a single sample at any time could indicate the ovarian reserve level. Indeed, there is some evidence that AMH level is correlated with several measurements of fertility (Mossa et al. 2017). The commercial use of AMH measurements as predictors of fertility remains largely unexplored.

The Horse

Fillies are often seen in oestrus during their second spring and summer (when they are yearlings), but under natural conditions it is unusual for them to foal until they are over 3 years old (see

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also Chapter 31). The mare is normally a seasonal breeder, with cyclic activity occurring from spring to autumn, whereas during the winter she will normally become anoestrus. However, it has been observed that some mares, especially those of native pony breeds, can cycle regularly throughout the year. This tendency can be enhanced if the mares are housed and given supplementary food when the weather is cold and if additional lighting is provided when the hours of daylight are short.

Horse breeding is influenced by the demands of thoroughbred racing because in the Northern hemisphere foals are aged from 1 January, irrespective of their actual birth date. As a result, the breeding season for mares has been, for over a century, determined by the authorities as running from 15 February to 1 July. Because the natural breeding season does not commence until about the middle of April with maximal ovarian activity from July onwards, it is obvious that the thoroughbred mare is often bred at a time when their fertility is suboptimal.

During winter anoestrus, both ovaries are typically small and bean-shaped, with dimensions of approximately 6 cm from pole to pole, 4 cm from the hilus to the free border, and 3 cm from side to side. It is not uncommon, however, in early spring or late autumn, for the anoestrous ovary to be medium to large in size and irregular in outline, due to the presence of numerous follicles of 10 to 15 mm diameter. Winter anoestrus is followed by a period of transition to regular cyclic activity. During this transition, the duration of oestrus may be irregular or very long, sometimes more than a month. The signs of oestrus during the transitional phase are often atypical, making it difficult for the observer to be certain of the mare's reproductive status. Also, before the first ovulation there is poor correlation between sexual behaviour and ovarian activity. Namely, oestrus in the transition period is often accompanied by the absence of large follicles, and some long spring oestruses are anovulatory. However, once ovulation has occurred, regular cycles usually follow.

The average length of the equine oestrous cycle is 20 to 23 days, with the cycles being longest in spring and shortest from June to September. The duration of oestrus is highly variable, lasting between 4 and 7 days. Ovulation occurs on the penultimate or last day of oestrus, and this relationship to the end of oestrus is fairly constant and irrespective of the duration of the cycle or the length of oestrus. Indeed, the manual rupture of the preovulatory follicle resulted in termination of oestrus within 24 hours (Hammond 1938). This makes predicting the time of ovulation, which is critical for the management of breeding by natural service or artificial insemination, potentially challenging. However, on the last day before ovulation, the tension in the preovulatory follicle (30-65 mm) usually subsides, and the palpable presence of a large fluctuating follicle is a sure sign of imminent ovulation. Thus the careful monitoring of the developing preovulatory follicle by transrectal ultrasonography can be performed to predict when ovulation is going to occur.

Ovulation of the preovulatory follicle results in the release of a single oocyte with a preponderance of ovulations from the left ovary. For example, Arthur (1958) recorded an incidence of 52.2% of ovulations from the left ovary from 792 postmortem reproductive tracts. In mares multiple ovulations commonly occur with a reported incidence of 27% (Burkhardt 1948), 18.5% to 37.5% (Arthur 1958), 21.5% (Henry et al. 1982) and 20.7% to 35.6% (Morel et al. 2005). The incidence is influenced by season and age; the majority of these are twin bilateral ovulations. It is also reported that there is a strong breed influence on twin ovulation rates, with thoroughbreds being prone, whereas with pony mares it is rare. All equine ovulations occur from the ovulation fossa only at the ovarian hilus. There may be the occasional protrusion of a CL but, because of the curvature of the ovary and the presence of the adjacent substantial fimbriae, these protrusions cannot be identified by rectal palpation.

The onset of oestrus typically occurs on the fifth through tenth day after foaling. This 'foal heat' is sometimes rather short, 2 to 4 days. It is traditional to cover a mare on the ninth day after foaling; however, the first two postparturient cycles are a few days longer than subsequent ones.

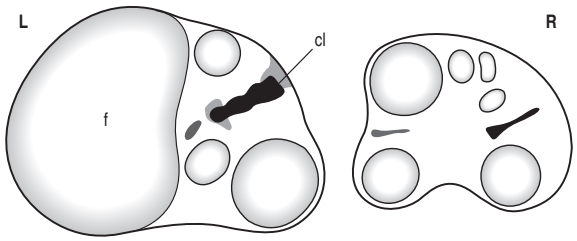
Folliculogenesis

There are large variations in ovarian size during the oestrous cycle depending on the number and size of the follicles. For example, during oestrus the ovary of the thoroughbred mare may contain two or even three follicles, each of 40 to 55 mm in diameter, and these, with other subordinate follicles, combine to give it a huge size. However, during dioestrus, the presence of an active CL and only atretic follicles result in the ovary being only a little larger than in anoestrus.

In the mare, follicular waves have been classified into: *major waves*, in which recruited antral follicles diverge into a dominant follicle and subordinate follicles in a similar manner to other monovular species; and *minor waves*, in which there is no divergence (Ginther & Bergfelt 1992). Major waves are further subdivided into primary waves, in which the wave originates middioestrus yielding a dominant follicle that ovulates, and secondary waves, in which the wave originates in late oestrus and either the dominant follicle is anovulatory or ovulation is delayed to after the end of oestrus (Ginther 1993). Minor wave and secondary waves occur most frequently during the transitional phase at the beginning of the breeding season, and the largest follicle does not attain more than 28 mm in size.

Day (1939), one of the early students of equine reproduction, produced a series of drawings of mare's ovaries collected after slaughter that give a clear picture of the changes that occur during the oestrous cycle (Figs. 1.3–1.7; they are half actual size) with the stage of the cycle having been determined clinically beforehand. Figs. 1.8 through 1.13 show examples of whole ovaries, crosssections, and B-mode ultrasound images.

Just before the onset of oestrus, several follicles enlarge to a size of 10 to 30 mm, with follicle deviation occurring when follicles reach around 22 mm in diameter. By the first day of oestrus, one follicle (the dominant follicle) is generally considerably larger than the remainder, having a diameter of 30 to 45 mm. During oestrus, this follicle will continue to grow at approximately 3 mm per day, until 2 days before ovulation when its growth plateaus at a diameter of 40 to 55 mm (Ginther 1993). The preovulatory



• **Fig. 1.3** Ovaries of a 5-year-old draft mare in oestrus. Note dominant, preovulatory follicle *(f)* in left ovary, 40 to 50 mm diameter, and regressing corpus luteum in the left ovary *(cl),* which was bright yellow in colour.

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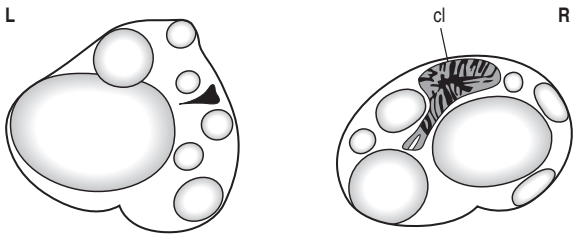
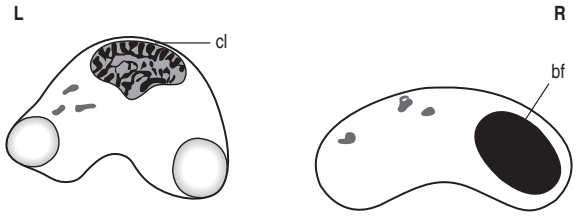
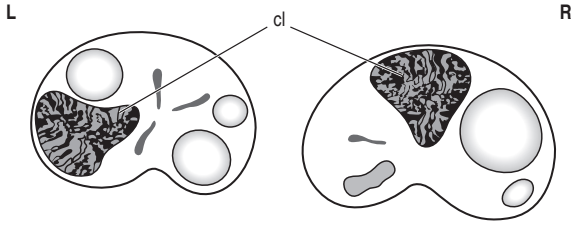


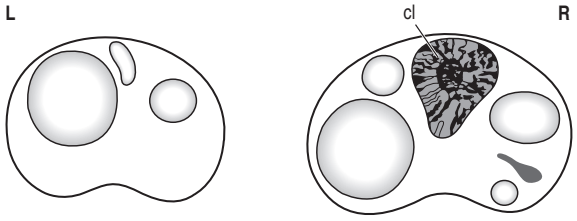
• **Fig. 1.4** Ovaries of a 9-year-old draft mare in dioestrus. Note presence of several sizeable follicles and corpus luteum *(cl)* in right ovary, which was orange in colour with the luteal tissue in loose pleats.



• **Fig. 1.5** Ovaries of a 4-year-old Shire mare in dioestrus. Corpus luteum *(cl)* in left ovary is brownish-red in colour with distinct pleats of luteal tissue. Right ovary contains a follicle filled with blood *(bf)*.



• **Fig. 1.6** Ovaries of a 6-year-old draught mare in dioestrus, with a corpus luteum *(cl)* in each ovary. Both are orange-yellow in colour with distinct pleats of luteal tissue.



• **Fig. 1.7** Ovaries of a 6-year-old hunter mare in dioestrus. Corpus luteum *(cl)* in right ovary is pale yellow in colour with distinct pleats of luteal tissue and a small central cavity.

follicle will continue to mature, and in the several hours before ovulation, it becomes much less tense and soft to touch. The horse is different from farm animal species in that it has not only much larger preovulatory follicles, but also that the follicles rupture from a specific indented region of the ovary termed the 'ovulation fossa'. The collapsed follicle is recognised by an indentation on the ovarian surface, and there is usually some haemorrhage into the follicle with the coagulum hardening within the next 24 hours. Quite frequently, the mare shows evidence of discomfort when the ovary is palpated soon after ovulation. Unless sequential transrectal palpation or ultrasonic examinations are performed, it is sometimes possible to confuse a mature follicle with the early corpus haemorrhagicum. This is because before ovulation, the follicular antrum is filled with follicular fluid, and then soon after ovulation it becomes filled with blood. For this reason, mares are sometimes incorrectly diagnosed as having failed to ovulate. For the next 3 days, the luteinising mass can be palpated as a resilient focus, but later it tends to have the same texture as the remainder of the ovary and is difficult to palpate. However, in pony mares Allen (1974) reported that it is possible to follow the growth of the CL by palpation because it forms a relatively large body within the small ovary of ponies. The CL enlarges within the ovary and attains maximum size after 4 to 5 days, but it does not protrude from the ovarian surface as in other species (Aurich 2011). After ovulation, the other follicles regress during the first 4 to 9 days of the ensuing dioestrus such that no follicles larger than 10 mm are likely to be present. On section of the ovary, it is brown and later yellow and of a triangular or conical shape, with the narrower end impinging on the ovulation fossa. Its centre is commonly occupied by a variable amount of dark brown fibrin. Plasma progesterone concentrations are maximal 8 days after ovulation, and the cyclical CL begins to regress on around day 14 of the cycle. Subsequently, there is a parallel fall in the blood progesterone concentration. From this day onwards, the events previously described recur. Ovulation with the subsequent formation of a CL does not always occur, and the follicle may regress or sometimes undergo luteinisation (Fig. 1.11B). These structures are termed haemorrhagic anovulatory follicles or luteinised unruptured follicles (Cuervo-Arango & Newcombe 2010).

Transrectal B-mode ultrasound imaging has been used to visualise follicles (Figs. 1.8–1.13). This is particularly useful in determining the timing of ovulation and also in detecting the possibility of twin ovulations. One particularly important observation is that in the preovulatory period there was a change in the shape of the follicle (from spherical to pear-shaped) and a thickening of the follicular wall, which, together with the assessment of the size of the follicle, could be used to predict the time of ovulation (Ginther et al. 2008). The examination of the CL after 5 days postovulation requires the use of ultrasonographic techniques. In approximately 50% of mares, there is persistence of the corpus haemorrhagicum as a central blood clot in the CL. Luteal activity strongly correlated with its vascularisation. Thus recent advances in the measurement of luteal blood flow using colour Doppler ultrasonography have enabled luteal functionality to be evaluated with reduced luteal blood flow indicating a regressing CL.

Reproductive Tract Changes During the Oestrous Cycle

The detection of the preovulatory period by visual examination of the vagina and the cervix using an illuminated speculum is possible. In dioestrus the vagina and cervix are pale pink in colour and the cervix is small, constricted and firm, while mucus is scanty and sticky. In contrast, during oestrus, there is a gradual increase in the vascularity of the genital tract and relaxation of the cervix with dilatation of the os. As oestrus progresses and ovulation approaches, the cervix becomes very relaxed and protrudes into the vagina; the folds are lying on the vaginal floor, with its folds oedematous. Additionally, the walls of the vagina glisten with clear, lubricant mucus. After ovulation there is a gradual reversion to the dioestrous appearance.

During anoestrus and pregnancy, both the vagina and cervix have a blanched colour; the cervix is narrow, constricted and generally turned away from the midline, the external os being filled with tenacious mucus.

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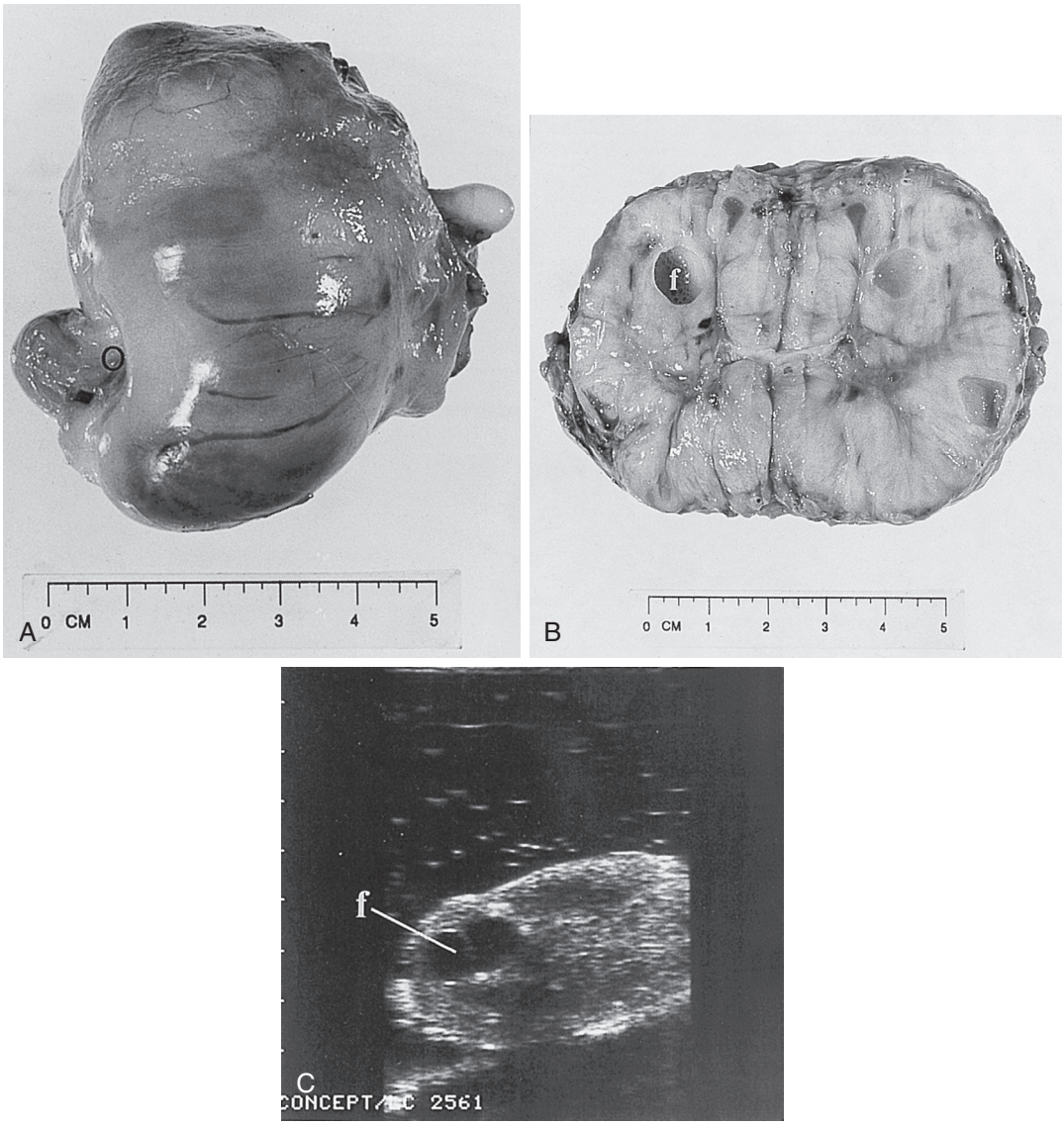

• **Fig. 1.8** Ovary from an acyclic (anoestrous) mare. (A) The ovary was hard on palpation with no evidence of follicular activity. Note the ovulation fossa *(o)*. (B) Crosssection of the ovary. Note that there are a few small follicles *(f)* 1 cm in diameter, which are contained within the ovarian matrix. (C) B-mode ultrasound image of the same ovary showing small anechoic (black) areas 1 cm in diameter, which are follicles *(f)*.

Cyclic changes can be detected during the palpation of the uterus per rectum. In the luteal phase under the influence of progesterone, the uterus increases in tone and thickness, but these features diminish when the CL regresses. At oestrus, there is no increase of tone as observed in other species (e.g., cow). During anoestrus and for the first few days after ovulation, the uterus is flaccid (Hammond & Wodzicki 1941).

Signs of Oestrus

The mare becomes restless and irritable, and she frequently adopts the micturition posture and voids urine with repeated exposure of the clitoris (Fig. 1.14). When introduced to a stallion or teaser, these postures are accentuated with the frequent raising of her tail to one side and leaning her hindquarters. The vulva is slightly oedematous, and there is a variable amount of mucoid discharge. A mare that is not in oestrus will usually violently oppose the advances of a stallion, and for this reason 'trying' mares at stud should be done over a gate, box door or stout fence. If the mare is in oestrus the stallion usually exhibits flehmen. Good stud management requires that a mare is accustomed to the procedure and that, because of the interval between the end of the last oestrus and the start of the next, she is teased 15 to 16 days after the end of the last oestrus (see Chapter 31).

Endocrine Changes During the Oestrous Cycle

The trends in endocrine changes are shown in Fig. 1.15. The secretion of FSH is biphasic with surges at approximately 10- to 12-day intervals. One surge occurs just after ovulation, with a second prominent surge in mid to late dioestrus, approximately 10 days before the next ovulation. This second and robust FSH surge occurs in the absence of an increase in LH. It has been suggested that this increase in FSH secretion, which is unique to the mare,

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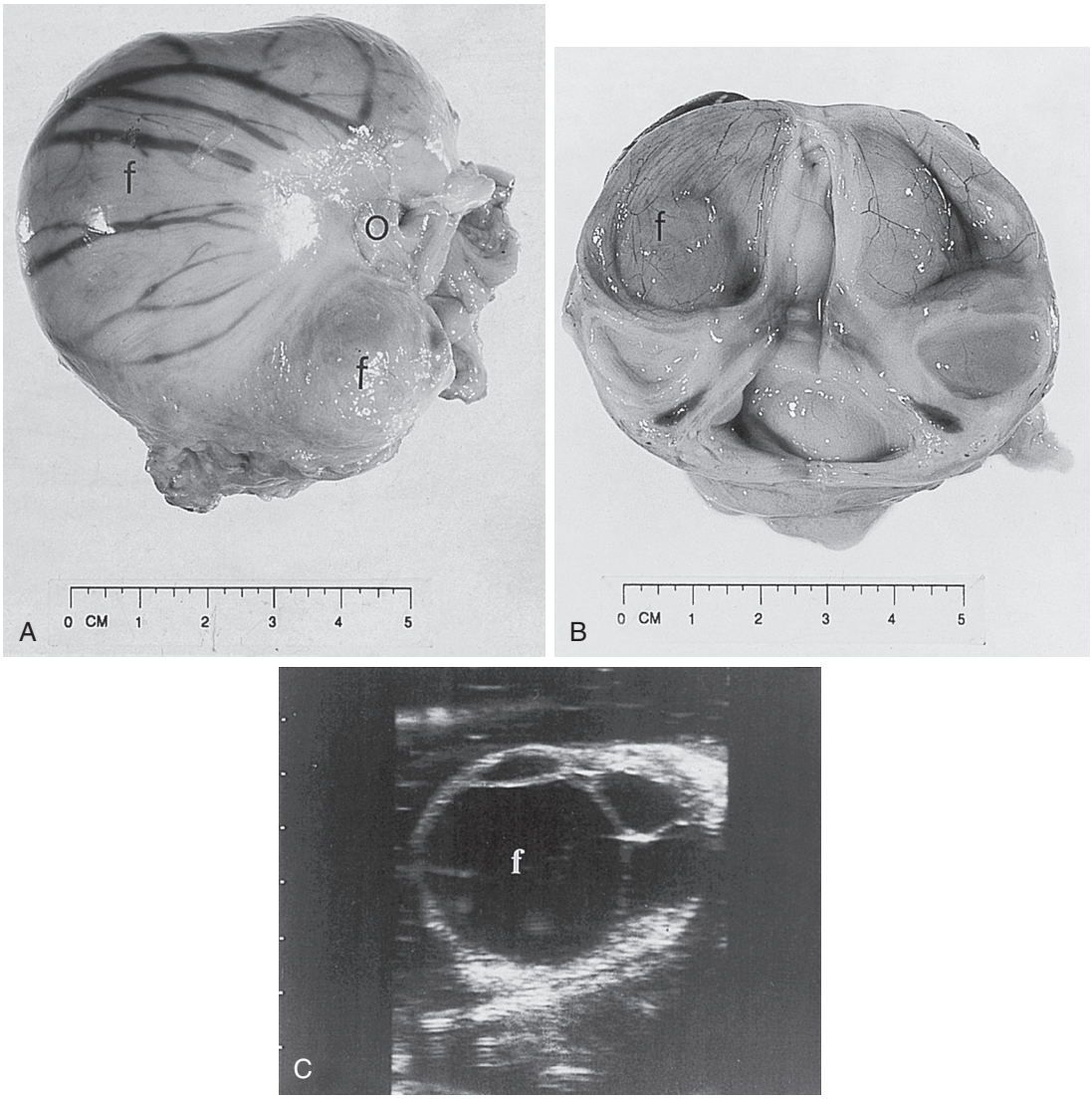

• **Fig. 1.9** Ovary from a mare in the early follicular phase. (A) The ovary was soft on palpation with evidence of large follicles near the surface of the ovary *(f)*. Note the ovulation fossa *(o)*. (B) Crosssection of the ovary. Note that three follicles are at least 2 cm in diameter. (C) B-mode ultrasound image of the same ovary showing one large anechoic (black) area about 3.5 cm in diameter, which is a follicle *(f),* together with three smaller ones.

is responsible for priming the development of a new follicular wave from which a follicle will ovulate at the next oestrus (Evans & Irvine 1975). Indeed, the increase in FSH is paralleled by an increase in the size of the largest follicle; however, after follicle deviation, peripheral FSH levels will start to decline. The pattern of LH secretion is also unusual in this species because there is no sudden surge of this hormone, but a gradual increase and persistence of elevated levels for 5 to 6 days on either side of ovulation. Maximal LH bioactivity occurs shortly before ovulation, with LH pulse increasing from 0.5 to a peak of 2 pulses per hour (Aurich 2011). Oestrogens in the peripheral circulation increase during oestrus and peak around ovulation before declining to basal levels during dioestrus. In contrast, concentrations of progesterone and other progestogens follow closely the physical changes of the CL. Specifically, there is an immediate increase in progesterone after ovulation, before reaching maximal concentrations on day 8 after ovulation. Thereafter, progesterone concentrations gradually decline, before a pronounced fall at the onset of luteolysis on approximately day 15 of the oestrous cycle. Luteolysis is induced by the endometrial secretion of $PGF_{2\alpha}$, which is in turn stimulated by oxytocin. In the mare there is no significant production of luteal oxytocin; instead, the source of the oxytocin is believed to be the posterior pituitary gland and the endometrial itself (Bae & Watson 2003).

The Cow

Cyclic Periodicity

Under normal conditions cattle are polyoestrous throughout the year. After puberty, cyclical activity will continue, except during pregnancy, for 3 to 6 weeks after calving, during periods of severe negative energy balance (as can be observed during high milk yield) and with a number of pathological conditions (see Chapters 22, 25 and 26). Some cows and heifers also fail to show overt signs of oestrus yet have normal cyclical activity, a condition referred to as 'silent heat', or suboestrus. However, this can be due to the

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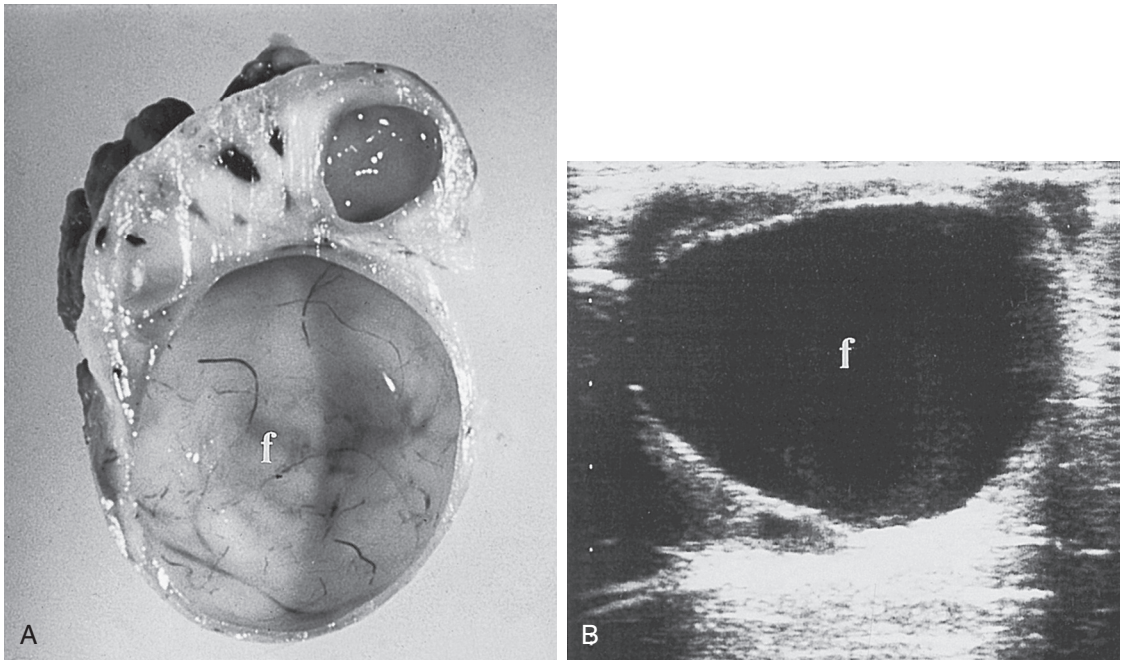
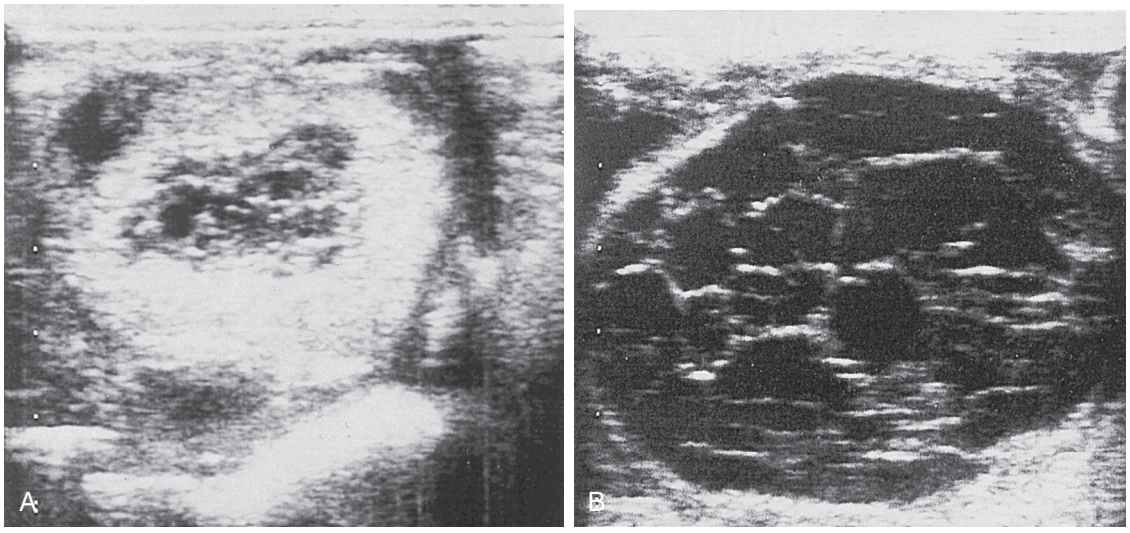


• **Fig. 1.10** Ovary of a mare with a single large preovulatory follicle. (A) Section of the ovary showing a 4 cm follicle *(f)*. (B) B-mode ultrasound image of a different ovary showing a 40 to 50 mm preovulatory follicle *(f)* as a large anechoic (black) area.



• **Fig. 1.11** (A) B-mode ultrasound image of an ovary showing the corpus haemorrhagicum. (B) B-mode ultrasound image of a 50 mm anovulatory follicle that is undergoing luteinisation.

herdsperson failing to observe the signs, rather than a failure of the cow to show signs.

The average length of the oestrous cycle is 20 days (range 18–22 days) in heifers and 21 days (range 18–24 days) in cows. Recent evidence indicates that the interservice interval is 22 days and that longer intervals are more widespread than originally thought (Remnant et al. 2015). For many years, the average duration of oestrus was recognised as being about 15 hours, with a wide range of 2 to 30 hours. However, there is good evidence that oestrus is much shorter in the modern Holstein cows, as compared to heifers, perhaps an average of 8 hours (Nebel et al. 2000, Dobson et al. 2008, Løvendahl & Chagunda 2010). This has been shown using radiofrequency data communication systems (e.g., 'HeatWatch') and is summarised in Table 1.1 (Nebel et al. 1997).

There are a number of factors that can influence the duration of oestrus: breed of animal, season of year, presence of a bull, nutrition, milk yield, lactation number, lameness, type of housing and the number of cows that are in oestrus at the same time (Wishart 1972, Esslemont & Bryant 1976, Roelofs et al. 2010). There is also good evidence that more signs of oestrus are observed during the hours of night, perhaps when the animals are least disturbed (Esslemont & Bryant 1976, Nebel et al. 2000). The optimal timing for artificial insemination (AI) is estimated to be 4 to 12 hours after of onset of oestrus (Roelofs et al. 2010), with spontaneous

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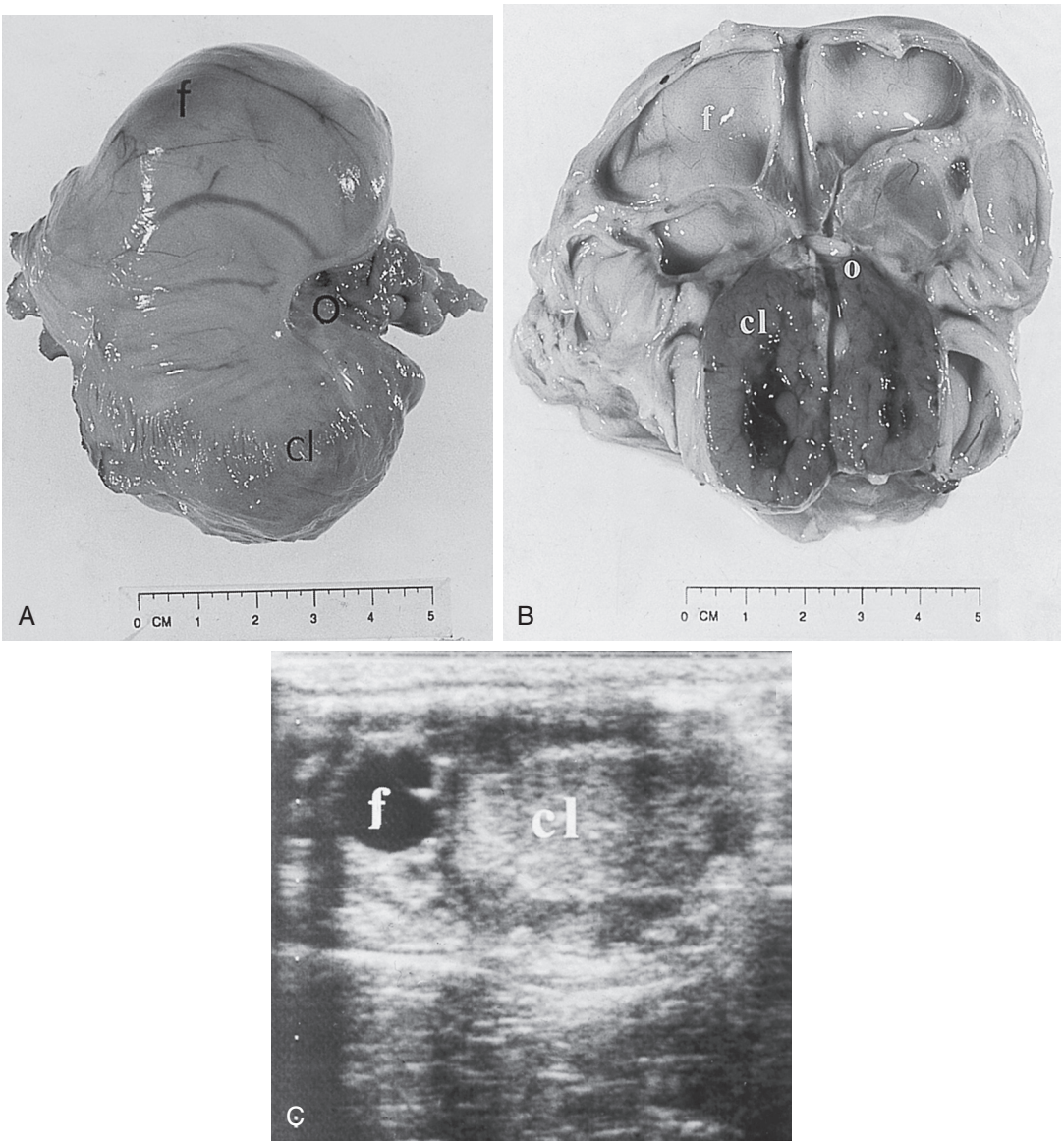

• **Fig. 1.12** Ovary of a mare in early dioestrus. (A) The corpus luteum *(cl),* although present, could not be palpated externally, whereas a follicle *(f)* could be identified. Note the ovulation fossa *(o)*. (B) Section of the same ovary. Note that the corpus luteum *(cl),* still with a central blood clot, impinges on the ovulation fossa *(o)* where ovulation occurred. Also, one large follicle *(f)* and several smaller ones can be identified. (C) B-mode ultrasound image of a different ovary showing the corpus luteum *(cl)* and follicles *(f)*.

ovulation occurring on approximately 28 to 30 hours after the onset of standing oestrus (or 12–18 hours after the end of oestrus) (Roelofs et al. 2005).

Signs of Oestrus

When AI is used, the accurate detection of oestrus by the herdsperson is paramount in ensuring optimum fertility. Poor detection is probably the most important reason affecting delayed breeding (Roelofs et al. 2010). Using traditional visual detection methods, the percentage of cows detected in oestrus varies from less than 50% to 90% of cows in oestrus (Roelofs et al. 2010). This can be further increased with the use of oestrus detection methods (e.g., pedometers, HeatWatch, physical methods) with rates of greater than 80% being reported using pedometers (Firk et al. 2002). However, on a number of commercial farms the detection of oestrus is a real challenge (see Chapters 22 and 25).

There are great variations among individual cattle in the intensity of oestrus signs, with the manifestations tending to be more marked in heifers than in cows. However, it is generally agreed that the most reliable criterion that a cow or heifer is in oestrus is that she will stand to be mounted by another (Williamson et al. 1972, Foote 1975).

The oestrous animal is restless and more active, and through the use of pedometer, there was an average increase in activity of two- to threefold at this time (Kiddy 1977, Lewis & Newman 1984). Furthermore, they showed that 75% of cows had peak pedometer readings on the day of onset of oestrus, whereas 25% peaked the day after oestrus. Equally, it has become clear that the use of pedometers requires them to be baselined for that individual

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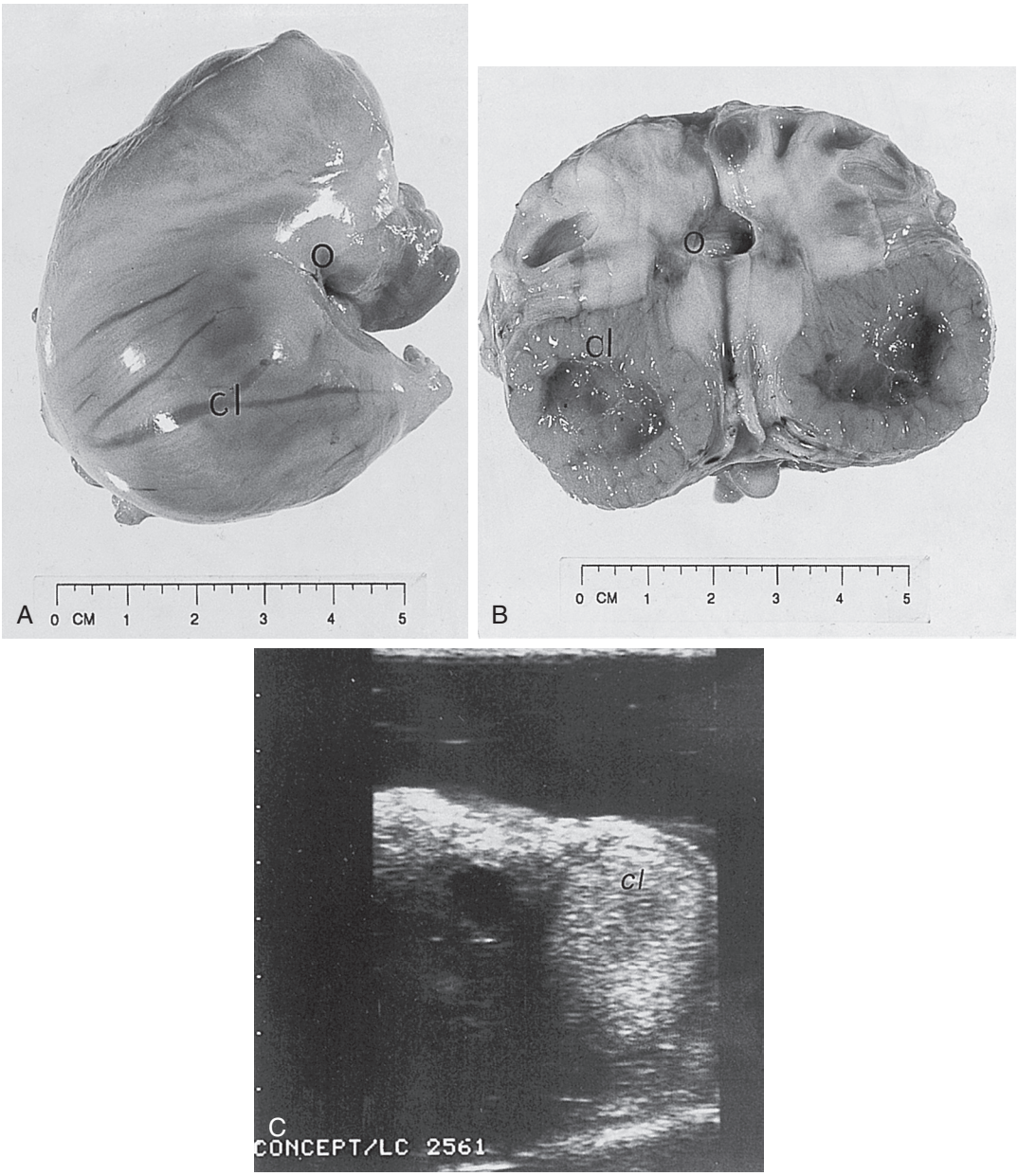

• **Fig. 1.13** Ovary of a mare in middioestrus. (A) The corpus luteum *(cl),* although present, could not be palpated externally there was no evidence of any follicles. Note the ovulation fossa *(o)*. (B) Section of the same ovary. Note the corpus luteum *(cl),* which impinges upon the ovulation fossa *(o)* where ovulation has occurred. (C) B-mode ultrasound image of the same ovary showing a speckled area corresponding to the corpus luteum *(cl)*.

animal (Roelofs et al. 2010). Cattle in oestrus tend to group with other sexually active individuals and spend less time resting and ruminating, which frequently leads to a reduction in milk yield. Indeed, reduced milk yield reliably indicated the onset of oestrus with a compensatory rebound at the next milking (Horrell et al. 1984). In this study of 73 dairy cows, when a cow produced 25% less milk, there was a 50% chance of her being in oestrus. On the rare occasions, that her yield fell by 75%, then oestrus was always present.

As the cow approaches oestrus, she tends to search for other cows in oestrus, and there is licking and sniffing of the perineum. During this period, especially during oestrus and just afterwards, the cow will attempt to mount other cows. Before she does this she will usually assess the receptivity of the other cows by resting her chin on their rump or loins. If the cow to be mounted is responsive and stands, she will mount and sometimes show evidence of pelvic thrusting (Esslemont & Bryant 1976). If the cow that is mounted is not in oestrus, she will walk away and frequently turn and butt the mounting cow. A positive mounting response lasts about 5 seconds (Hurnik et al. 1975); however, if both cows are in oestrus, then this response increases to about 7 seconds. There is considerable variation in the number of standing events during oestrus between individuals and can vary from 1 to over 50 (Hurnik et al. 1975, Roelofs et al. 2005) (Table 1.1).

In some animals, during oestrus there is a vulvar discharge of transparent mucus with its elasticity causing it to hang in complete,

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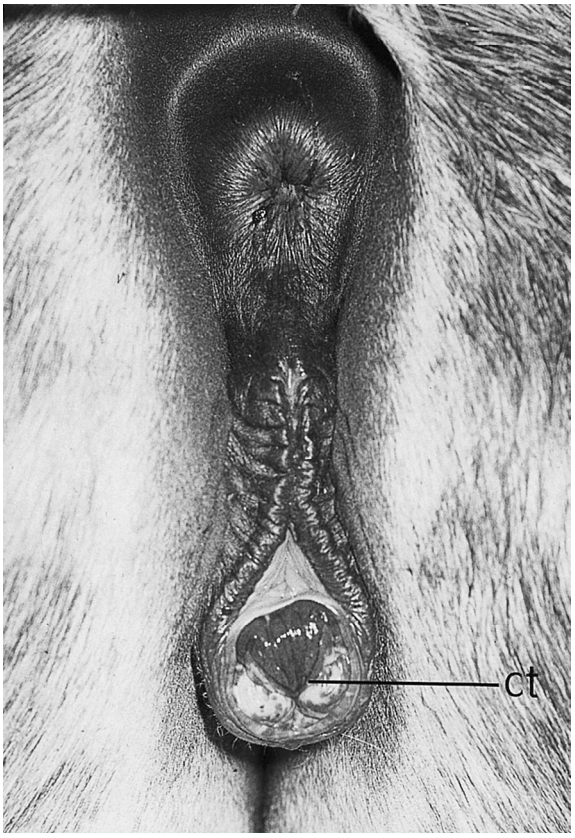
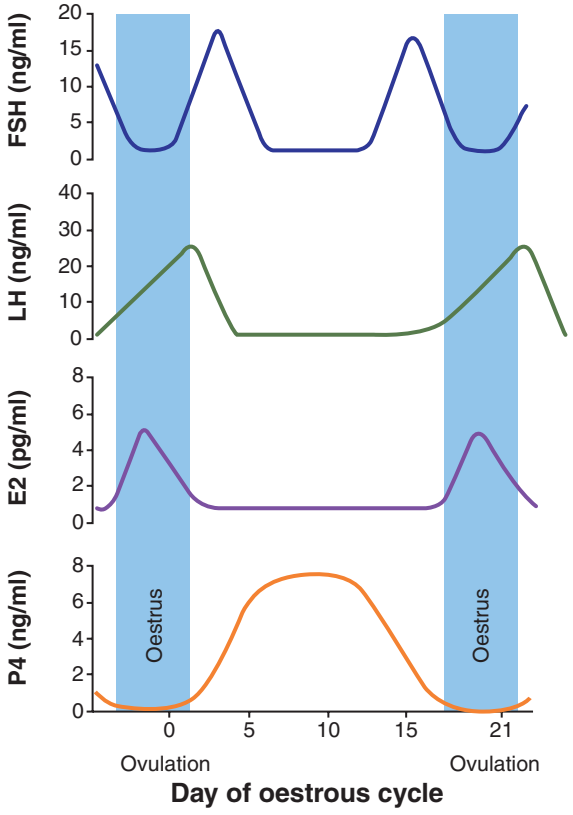


• **Fig. 1.14** Exposure of the clitoris *(ct)* in response to teasing.



• **Fig. 1.15** Trends in hormonal concentrations in the peripheral circulation of the mare during the oestrous cycle.

TABLE 1.1 Duration of oestrus and number of standing events (mean and standard deviation) as determined by the 'heatwatch' oestrus detection system

	NULLIPAROUS HEIFERS		PLURIPAROUS COWS	
	Holstein	Jersey	Holstein	Jersey
No. of animals	114	46	307	128
Duration of oestrus (hours)	11.3±6.9	13.9±6.1	7.3±7.2	7.8±5.4
No. of standing events	18.8±12.8	30.4±17.3	7.2±7.2	9.6±7.4

From Nebel et al. 1997.

clear strands from the vulva to the ground. It also can adhere to the tail and flanks. The vulva may be slightly swollen and congested, and the tail may be slightly raised. The hair of the tailhead is often ruffled, and the skin sometimes becomes excoriated through mounting by other cows. For the same reason, the rear of the animal may be soiled with mud. If at pasture, the oestrous cow may wander from the herd; if isolated, she will bellow. When she has access to a bull, the two animals lick each other, and the cow often mounts the bull before standing to be mounted by him. For a short time after mating, the cow will stand with a raised tail and arched back; if the actual service had been missed, then this posture would indicate that mating had occurred.

The body temperature of dairy cows falls by about 0.5°C on the day before oestrus; then it increases during oestrus and falls by about 0.4°C at ovulation. However, the vaginal temperature of 37.7°C was lowest on the day before oestrus, increased by 0.2°C on the day of oestrus and increased for the next 6 days until a plateau was reached. This was followed by a gradual decline from 7 days before the next oestrus (Lewis & Newman 1984). However, practical detection of this is tedious, but the use of temperature data logger attached to a vaginal implant device may enable such measurements to be made (Fisher et al. 2008, Sakatani et al. 2016). Automated methods of measuring the related increase in milk temperature in the milking parlour have also been described (Maatje & Rossing 1976); however, these milk-based methods often come with high false positive results.

The pH of the vagina also fluctuates throughout the oestrous cycle and is lowest, at approximately pH 7.3 on the day of oestrus (Lewis & Newman 1984).

Within 2 days of service, there is an occasional yellowish white vulvar discharge of mucus containing neutrophil leukocytes from the uterus. At about 48 hours after oestrus, irrespective of being served, heifers and many cows show a bright red sanguineous discharge, with the blood coming mainly from the uterine caruncles.

Cyclic Changes in the Vagina

The main variations are in the epithelial cells of the anterior vagina and in the secretory function of the cervical glands. During oestrus, the anterior vaginal epithelium becomes greatly thickened as a result of cell division and the growth of tall, columnar, mucus-secreting superficial cells. Leukocytic invasion of the vaginal mucosa is maximal 2 to 5 days after oestrus. During dioestrus the epithelial

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cells vary from flat to low columnar (Hammond 1927, Blazquez et al. 1989). Copious secretion of mucus by the cervix and anterior vagina begins a day or so before oestrus, which increases during oestrus and then gradually diminishes; it is absent by the fourth day after oestrus. The mucus is transparent and flows readily.

At the same time, the crystallisation pattern of the cervical mucus varies. These can be observed when dried smears of mucus are examined microscopically. During oestrus and for a few days afterwards, the crystals are disposed in a distinct arborisation pattern, whereas for the remainder of the cycle this pattern is absent. This phenomenon, together with the character and amount of cervical mucus, is dependent on the elevated peripheral oestrogen concentration. The postoestrous vaginal mucus shows floccules composed of leukocytes and, as previously mentioned, blood is frequently present.

The hyperaemia of the vaginal and cervical mucosae is progressive during prooestrus and oestrus; the vaginal protrusion of the cervix is tumefied and relaxed such that one or two fingers can be inserted into the cervical os. During metoestrus, there is a rapid reduction in vascularity, and from 3 to 5 days after oestrus the mucosa is pale and quiescent. At the same time, the external os is constricted, and its mucus becomes scanty, sticky and pale yellow or brown. There are also cyclic variations in vaginal thermal conductance and vaginal pH. Namely, there is an increase in vaginal thermal conductance just before oestrus (Abrams et al. 1975). When pH electrodes were placed in the cervical end of the vagina, the pH fell from 7.0 to 6.7 one day before the first signs of oestrus, before decreasing further to pH 6.5 at the start of oestrus (Schilling & Zust 1968). Similarly, vaginal electrical resistance increased during oestrus (Smith et al. 1989). This can be measured using commercially available devices such as Ovascan or Ovatec (Zuluaga et al. 2008, Hockey et al. 2010).

Cyclic Changes in the Uterus

During oestrus the uterus becomes congested, and the endometrium is suffused with oedematous fluid causing its surface to glisten. The muscularis layer is physiologically contractile such that when the uterus is palpated per rectum, this muscular irritability, coupled with the marked vascularity, conveys a highly characteristic tonic turgidity to the palpating fingers. Hence the horns will feel erect and coiled. This tonicity is present on the day before and through to the day after oestrus but is at its maximum during oestrus. With experience the veterinarian can detect oestrus on this sign alone. Between 24 and 48 hours after oestrus, the uterine caruncles show petechial haemorrhage, which gives rise to the postoestrous vaginal discharge of blood. In heifers there are often also associated perimetrial subserous petechiae. During dioestrus the endometrium is covered by a scanty secretion from the uterine glands. Quantification of the morphometric characteristics of the bovine endometrium during the oestrous cycle revealed that the total endometrial area increased during oestrous, whereas the density of the glands increased under the influence of progesterone during the luteal phase (Wang et al. 2007).

Cyclic Changes in the Ovaries

The size and contour of the ovaries will depend on the stage of the cycle. Postmortem sections and transrectal ultrasonography of such ovaries has revealed the most significant structures to be Graafian follicles and CLs. Usually one follicle ovulates, and hence one oocyte is liberated after each oestrus. Classically, the incidence of double ovulations in cows was considered to be 4% to 5% of cows, with triplet ovulations occurring more rarely. However, more recent evidence in the high-producing dairy cow indicates that the double ovulation incidence is more in the range of 13% to 20%. The incidence is greater in multiparous cows compared with primiparous cows. The influence of milk yield remains equivocal (López-Gatius et al. 2005, Lopez et al. 2005) with high incidence (>20%) reported in nonlactating cows (Mann et al. 2007). In dairy cattle about 60% of ovulations are from the right ovary, although in beef cattle the functional disparity between the ovaries is not great.

Follicular Growth and Development

For the purposes of clarity, this section will focus on the mature, unbred heifer. Follicular growth and atresia throughout the oestrous cycle of cattle has been extensively studied (Adams et al. 2008, Scaramuzzi et al. 2011, Ginther 2016). In the studies of Bane and Rajakoski (1961), two waves of growth were demonstrated, with the first wave beginning shortly after ovulation and the second starting in the midluteal phase. Consequently, a dominant follicle of 9 to 14 mm was present from day 5 to 11 of the cycle before becoming atretic. Then, in the second wave the ovulatory follicle developed between day 15 and 20 and was 9 to 16 mm in size. Thus this preovulatory follicle is selected 3 to 5 days before ovulation (Pierson and Ginther 1988). Subsequent studies have demonstrated that there are three, as well as two waves of follicular development (Sirois & Fortune 1988, Savio et al. 1990). In the case of three-wave follicular patterns, waves emerge on days 1, 8 and 16 of the cycle, whereas in two-wave patterns they emerge on day 1 and days 9 to 10 of the cycle. In each case the dominant follicle will undergo atresia if under the influence of progesterone. However, if there is an active dominant follicle present when luteolysis is induced, then this will become the ovulatory follicle. There are reports of majority of either two- or three-wave per cycle; however, others have reported a more equal distribution (Adams et al. 2008). In a small number of cows, four-wave cycles have been observed, although this is usually associated with delayed luteolysis and an extended interoestrous interval of more than 24 days. In contrast, four waves are common in *Bos indicus* cows (Bo et al. 2003) (Chapter 28).

The most notable feature is the regularity of the number of waves of follicular growth per oestrous cycle, which probably reflects genetic or environmental influences. Indeed, the proportion of cows with a repeatable number of waves per cycle within an individual (70%) was twofold greater than those individuals that had alternating patterns (30%) (Adams et al. 2008). Those individuals with two waves per cycle tend to have shorter interoestrous intervals (19.8 days) and larger preovulatory follicles than those animals with three waves (22.5 days). Interesting data (Tables 1.2 and 1.3) was obtained by Sartori et al. (2004) using sequential transrectal ultrasonography and shows some of the effects of parity and/or lactation on folliculogenesis, ovulation and CL formation, as well as serum oestrogen and progesterone concentrations. A noticeable feature is the high number of multiple ovulations in cows (17.9%) compared with heifers (1.9%). In addition, cows had larger ovulatory follicles and luteal tissue mass, but these structures were associated with lower oestrogen and progesterone concentrations, respectively.

The follicular waves are initiated by a small rise in circulatory FSH; if this does not occur or is delayed, the follicular wave also does not occur or is delayed. There is evidence that the follicle that is destined to become the dominant one may be slightly larger and

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TABLE 1.2 Follicular waves and multiple ovulations in nulliparous heifers ($n = 27$) and lactating cows ($n = 14$) (The data are mean ± SEM.)

	Heifers	Cows
Interovulatory interval (days)	22.0±0.4	22.9±0.7
Percentage with two follicular waves/cycle	55.6	78.6
Percentage with three or more follicular waves/cycle	44.4	21.4
Days from luteolysis to ovulation	4.6±0.1	5.2±0.2*
Multiple ovulation rate (%)	1.9	17.9*

Adapted from Sartori et al. 2004.
*P<0.05 vs heifers

TABLE 1.3 Comparisons of data (mean ± SEM) obtained from Holstein heifers and lactating cows with single follicle dominance and ovulation

	Heifers	Cows
Size of ovulatory follicle at luteolysis (mm)	11.0 ± 0.5	13.1 ± 0.7[a]
Maximum size of ovulatory follicle (mm)	15.0 ± 0.2	17.2 ± 0.5[b]
Maximum serum oestradiol concentration before ovulation (pg/ml)	11.4 ± 0.6	7.3 ± 0.8[b]
Maximum luteal tissue volume (mm^3)	7303 ± 308	11248 ± 776[b]
Maximum serum progesterone concentration (ng/ml)	7.3 ± 0.4	5.8 ± 0.6[a]

Adapted from Sartori et al. 2004.
[a]P<0.05 vs heifers.
[b]P<0.001 vs heifers.

have a better blood supply than the rest (Zeleznik et al. 1981), such that it responds to the rise in FSH and produces more oestradiol and inhibin, which are critical for being selected for dominance (Scaramuzzi et al. 2011). There is also good evidence that the IGF system plays a critical role in the selection and growth of the dominant follicle in the cow. The IGFs stimulate granulosa cell growth and synergise with FSH to stimulate oestradiol production (see reviews by Fortune et al. (2001) and Mazerbourg et al. (2003)).

This means that during dioestrus several large follicles will be found ranging in size from 7 to 15 mm in diameter. These follicles do not alter the general oval contours of the ovaries, but do cause some overall variation in gross ovarian size. The ease of palpating them transrectally will depend upon the size, degree of protrusion and relationship with the CL. During prooestrus and oestrus, the dominant follicle destined to ovulate enlarges, and ovulation occurs when it has attained a size of 12 to 20 mm (Fortune 1994, Ginther et al. 1996, Adams et al. 2008). On transrectal palpation of the ovaries during oestrus, it is sometimes possible to detect the ovulatory follicle as a slightly bulging, smooth, soft area on the ovarian surface, although it is readily identified using transrectal ultrasonography. Ovulation may occur from any aspect of the ovarian surface, and the subsequent shape of the ovary when the CL develops will be influenced chiefly by this site. The point of ovulation is usually an avascular area of the follicular wall; consequently, haemorrhage is not a feature of bovine ovulation, although there is marked postovulatory congestion around the rupture point and sometimes a small blood clot is present in the centre of the new CL.

The Corpus Luteum of the Oestrous Cycle

After rupture the oocyte is expelled through a small breach in the surface of the follicle, and after the escape of the greater part of its fluid, the follicle collapses. If the opportunity arose for repeatedly carrying out rectal examinations during oestrus and for the subsequent 36 hours, this collapse would be detected. The ovary also frequently feels flattened and soft. However, on a single examination, the detection of the collapsed follicle is challenging, and the ovary can be classified as having no distinctive structure. If such an ovary is examined postmortem, the surface from which ovulation has occurred is wrinkled and distinctively bloodstained. The ovulatory point can also be observed (Ireland et al. 1980, Woad & Robinson 2016). The CL develops by hypertrophy and luteinisation of the granulosa and thecal cells that lined the follicle. Enlargement is extremely rapid, such that within 2 days it will be approximately 10 mm in diameter (Prokopiou et al. 2014). At this stage the developing CL is soft and yields on palpation; it is light brown in colour and the luteinised cells form loose pleats. The CL attains its maximum size by the day 7 or 8 of the cycle (Fig. 1.16). The luteinised pleats are now relatively compact, and the body comprises a more or less homogeneous mass that is orange-yellow in colour. Its shape varies; the majority are oval, but some are irregularly square or rectangular. The greatest dimension of the fully developed structure varies, but typically it is 20 to 25 mm. The changes in the CL dimensions are shown in Fig. 1.17. The weight of a fully developed CL also varies from 4.1 to 7.4 g; sometimes the centre of the CL is occupied by a cavity (Fig. 1.18, 1.28). Such cavities are seen in up to 50% of the CLs collected at abattoirs and are filled with yellow fluid. The size of the cavity varies, but it is small in the majority (approximately 4 mm in diameter). However, occasionally it is larger, up to 10 mm or more (Kastelic et al. 1990). In these cases there is evidence of ovulation as shown by the presence of a pinhead depression in the centre of the CL that projects from the ovarian surface. This serves to differentiate the CL from an abnormality of the cow's ovary in which the follicular wall is luteinised in the absence of ovulation to form a pathological structure, namely a luteal cyst (see Chapter 22). Nevertheless, it is probable that cavity-filled CL were described in the past as a cystic corpus luteum and regarded as pathological; the presence of a cavity in a CL is normal.

Projection of the Corpus Luteum From the Surface of the Ovary

As the CL enlarges, it tends to protrude from the ovary, thus stretching the serosa that covers the surface of the ovary, such that when it attains its maximum size, it often forms a distinct projection. The degree and appearance of this projection vary. In the majority it is a distinct bulge about 10 mm in diameter with a clear-cut constriction where it joins the general contour of the ovary. In other cases it is nipple-like (Fig. 1.16). In a third type, the projection is indistinct, and in this case the CL can occupy the greater part of the ovary. It has been suggested that the type of protrusion that develops depends on the extent of the

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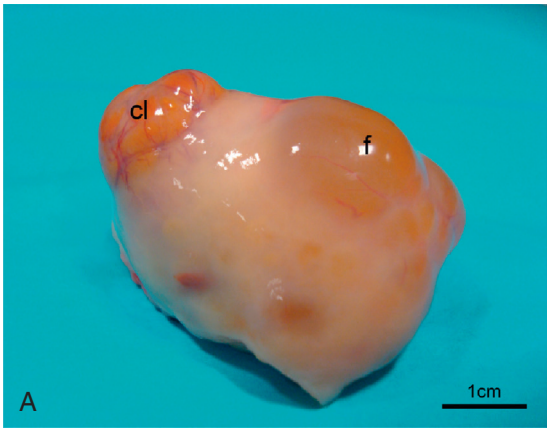
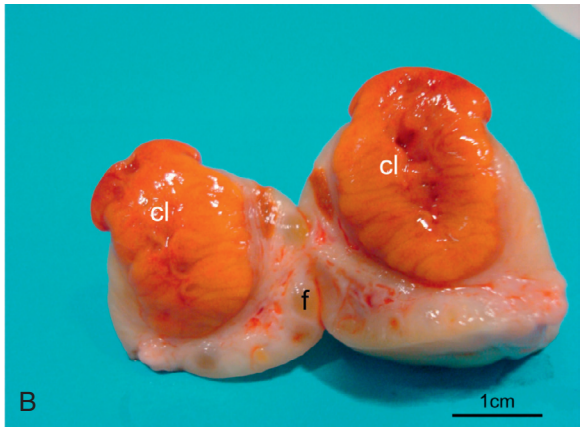
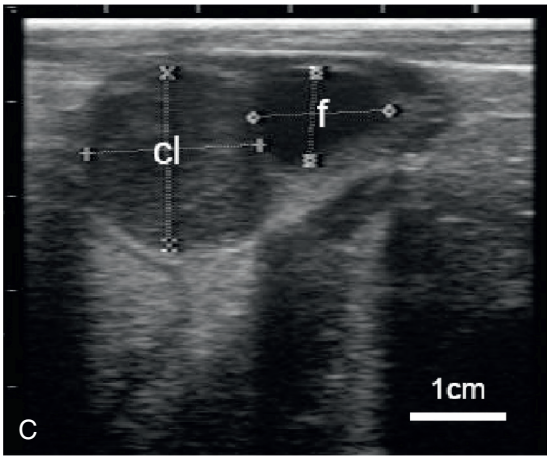


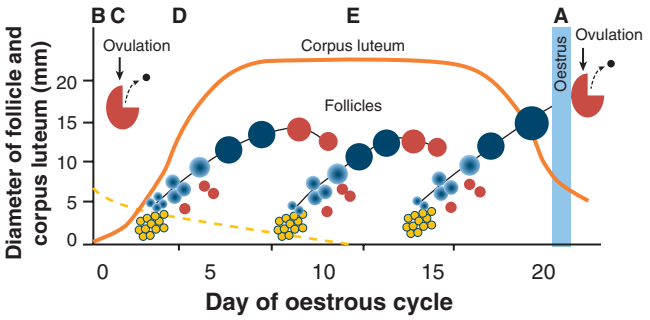




• **Fig. 1.16** Ovary of cow in late dioestrus. (A) A mature corpus luteum *(cl)* with ovulation papilla could be readily palpated, together with a large antral follicle *(f)*. (B) Section of an ovary showing a developing corpus luteum *(cl)* and small antral follicle *(f)*. (C) B-mode ultrasound image of the same ovary showing a speckled area corresponding to the corpus luteum *(cl)* and the midcycle follicle *(f)*.

surface of the ovary occupied by the follicle just before ovulation. Figs. 1.19 through 1.27 show bovine ovaries during the oestrous cycle[1].

[1]Throughout the book, drawings of bovine ovaries are of a section from the attached to the free border through the poles. In those cases in which this section did not pass through the greatest dimension of the significant corpus luteum or the largest follicle, the drawing has been made as though it did so, but without materially altering the size of the ovary.



• **Fig. 1.17** The development of follicular waves *(dotted line)* and corpus luteum *(solid line)* during the oestrous cycle of the cow. (A–E refers to Fig. 1.19–1.27.)

Regressing Corpus Luteum

The CL maintains its maximum size and remains unaltered in appearance until luteolysis and the onset of prooestrus. From this point it undergoes a rapid reduction in size and changes in colour and appearance. By the middle of oestrus its diameter is reduced to 15 mm, and its protrusion is much smaller and less distinct. Its colour also changes to a very bright yellow (hence the name corpus luteum: the yellow body). This colour contrasts strikingly with the orange-yellow colour of the active structure. Its consistency becomes compact and dense; scar tissue invasion will commence. By the second day of next dioestrus, its size is reduced to about 10 mm, and its outline is becoming irregular. By this time its colour is changing to brown. By the middle of dioestrus, it has shrunk to about 5 mm, and its surface protrusion is a little larger than a pinhead. As it further regresses, its colour tends to change to red or scarlet; small red remnants of CLs tend to persist for several months.

Size of the Ovaries

From the foregoing account it will be appreciated that the size of an ovary will depend chiefly on the stage in the oestrous cycle at which it is examined and on whether or not it contains an active CL. The presence of follicles does not alter the size of an ovary to anything like the same extent. In the great majority of heifers and young cows examined at any time between day 6 and 18 of the cycle, one ovary will be distinctly larger than the other. In heifers the approximate dimensions of the larger one will be 3.5 cm from pole to pole, 3 cm from the attached to the free border and 2.8 cm from side to side. (All ovarian dimensions given in this book are in this order.) The CL will protrude from some part of its surface, although the degree of protrusion will vary between ovaries. The smaller ovary will have approximate dimensions of 2.5 × 1.5 × 1.2 cm, and it will be flat from side to side.

During the first 4 or 5 days after ovulation, there will be relatively little variation in size, for as yet the developing CL has not attained a sufficient mass to influence the size of the ovary, although the regressing CL will be smaller. During oestrus there will also be little difference in size unless the ovary which contains the developing preovulatory follicle also contains the regressing CL. In this case the ovary containing the two structures will be a little larger than the other, but not strikingly so.

Ovaries of the Multiparous Cow

The ovaries of the normal multiparous cow do not differ greatly from those of the heifer or first calver. However, they tend in

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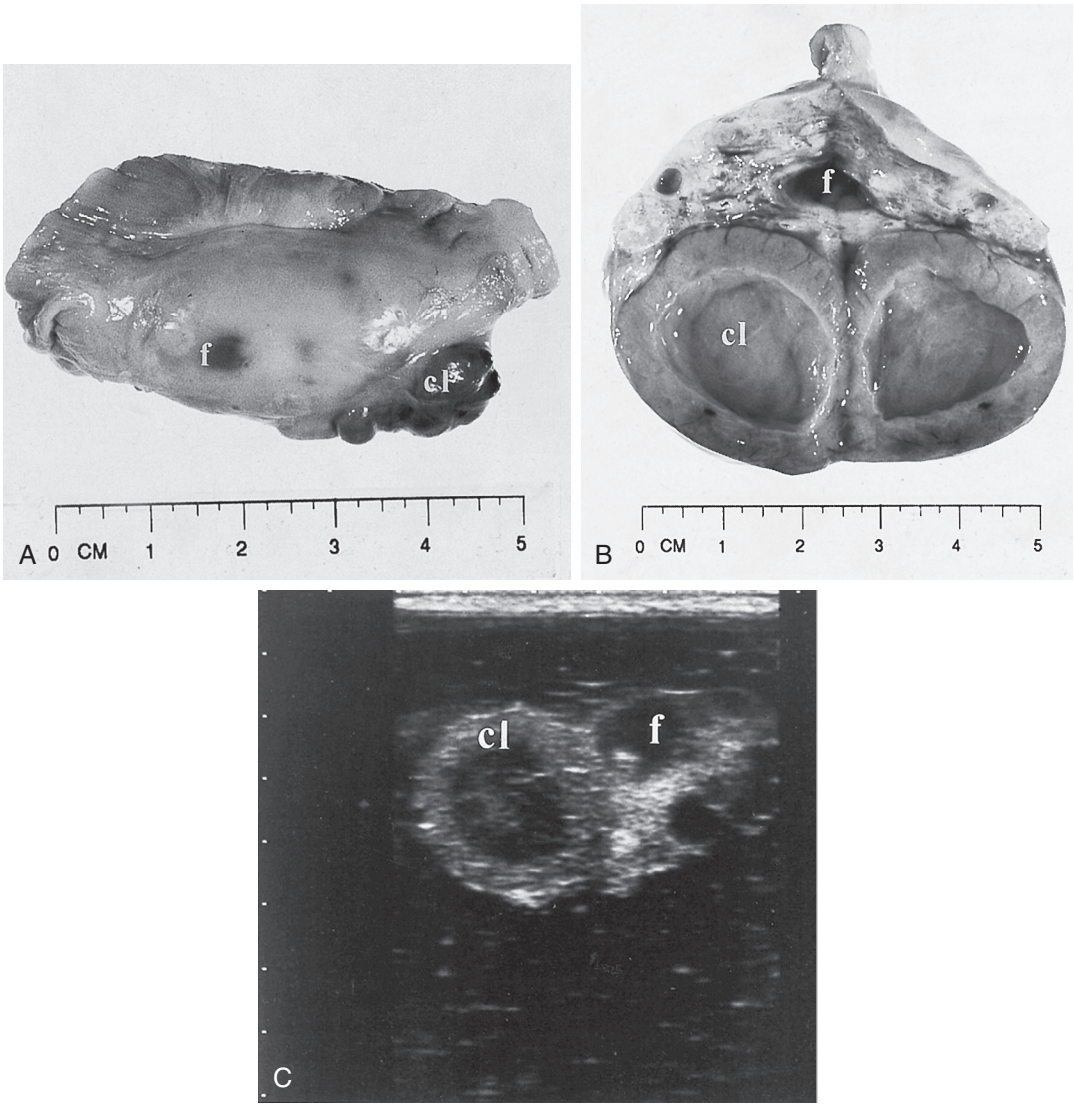
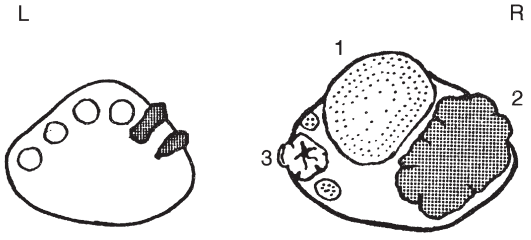
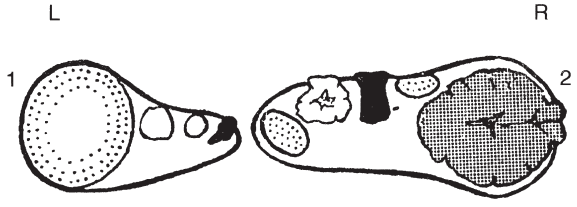


• **Fig. 1.18** Ovary of cow in middioestrus. (A) A mature corpus luteum *(cl)* with prominent ovulation papilla and midcycle follicle *(f)*. (B) Section of the same ovary showing the corpus luteum *(cl)* with a central lacuna that was filled with orange-yellow fluid and the midcycle follicle *(f)*. Note that the 'wall' of the corpus luteum comprises at least 5 mm thickness of luteal tissue. (C) B-mode ultrasound image of the same ovary showing a speckled area corresponding to the 'wall' of the corpus luteum *(cl),* the central lacuna and also the midcycle follicle *(f)*.



• **Fig. 1.19** Ovaries of a first calf heifer in oestrus. *(1)* Ovulatory follicle; *(2)* regressing corpus luteum, bright yellow; *(3)* corpus albicans. Stage A in Fig. 1.17.



• **Fig. 1.20** Ovaries of a first calf heifer in oestrus. *(1)* Ovulatory follicle; *(2)* regressing corpus luteum, brick-brown in colour. Stage A in Fig. 1.17.

many cases to be larger. This increase in size is due in part to the progressive deposition of scar tissue resulting from prolonged functional activity and in some cases also to the presence of large numbers of small but visible follicles. Typically, the ovary that does not contain a CL measures 4 × 3 × 2 cm. Nevertheless, it is generally possible in middioestrus to detect the CL, which even without a distinct protrusion gives the ovary a plum-like shape, whereas the other is distinctly flattened from side to side. As well as normal follicles and CLs at various stages of development and regression, there are additional structures, namely old scarred CLs of previous pregnancies. They generally show as a white, pinhead-sized projection on the ovarian surface of the ovary and

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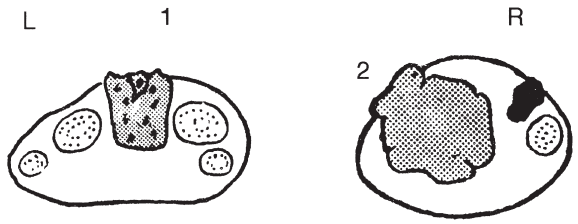
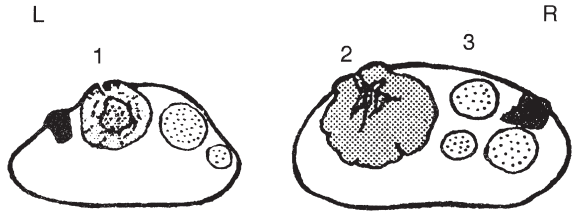
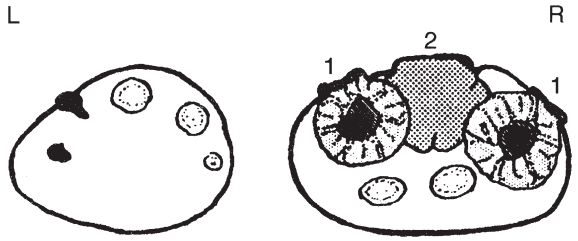


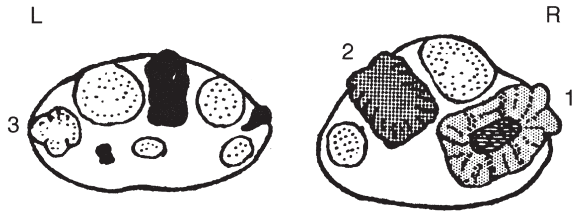
• **Fig. 1.21** Ovaries of a nulliparous heifer just after ovulation. *(1)* Collapsed follicle, surface wrinkled and bloodstained petechiae present in wall; *(2)* regressing corpus luteum, bright yellow. Stage B in Fig. 1.17.



• **Fig. 1.22** Ovaries of a young cow 1 day after ovulation. *(1)* Developing corpus luteum, with loose pleats, colour is pale cream, central cavity; *(2)* regressing corpus luteum, bright orange-yellow centre filled by connective tissue; *(3)* corpus albicans. Stage B in Fig. 1.17.

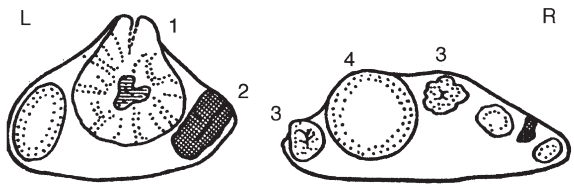


• **Fig. 1.23** Ovaries of a young cow 2 days after ovulation. *(1)* Twin corpora lutea, some haemorrhage; *(2)* regressing corpus luteum, bright yellow. Stage C in Fig. 1.17.

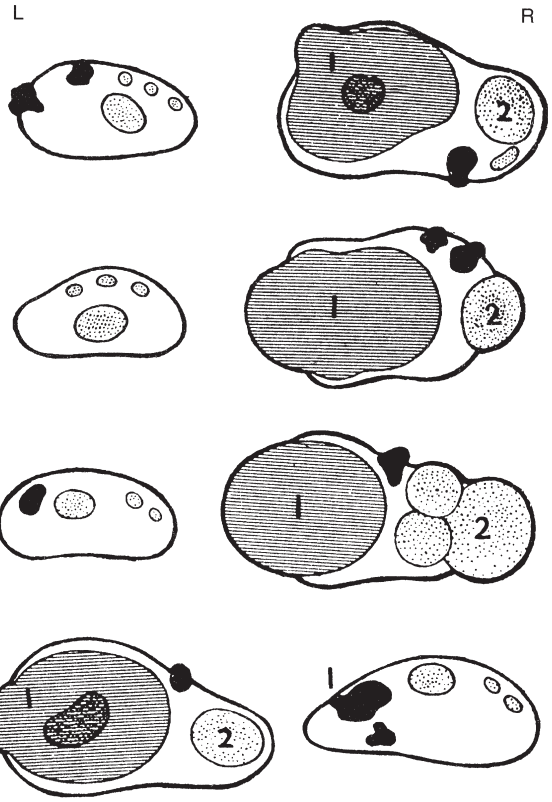


• **Fig. 1.24** Ovaries of a 4-year-old cow in early dioestrus. *(1)* Active corpus luteum, loose pleats, colour orange-yellow, central cavity; *(2)* regressing corpus luteum, dense, and brown colour; *(3)* corpus albicans. Stage C in Fig. 1.17.

are comprised mainly of scar tissue. They are irregular in outline, with a maximum dimension of 5 mm. They are brownish white or white in colour; hence they are called corpus albicans (white body). The CL of pregnancy does not atrophy after parturition as quickly as the CL from the oestrous cycle. It is an appreciable structure for several weeks after parturition, brown in colour and about 10 mm in diameter. It becomes progressively invaded by scar tissue, but remains throughout the cow's life. On postmortem examination the presence of the corpus albicans serves to distinguish the cow from the heifer; in the cow a count of the number of corpora albicantia gives the number of calves born.



• **Fig. 1.25** Ovaries of a 6-year-old cow in early dioestrus. *(1)* Active corpus luteum, orange-yellow, atypical protrusion; *(2)* regressing corpus luteum, small, shrunken, scarlet colour; *(3)* corpus albicans; *(4)* follicle. Stage D in Fig. 1.17.



• **Fig. 1.26** Ovaries of nulliparous heifers in dioestrus. *(1)* Corpus luteum; *(2)* largest follicle. Stage E in Fig. 1.17.

The fully developed corpus luteum is present by day 7 post ovulation and persists unchanged until the onset of prooestrus. Exceptional corpora lutea are shown in Fig. 1.28.

Ultrasonic Appearance of the Ovaries

In previous sections of this chapter, there are descriptions of the texture (as determined by palpation) and the appearance (as determined by sectioning after slaughter) of the ovaries and their contents. The advent of transrectal B-mode, real-time, grey scale ultrasound imaging, particularly using a 7.5 to 10 MHz transducer, has enabled detailed, accurate, sequential examination of the ovaries to be made without adversely affecting the cow's health or fertility. For a detailed description of the echogenic appearance of the ovaries, readers are advised to consult Pierson and Ginther (1984) and DesCôteaux et al. (2009).

The following normal structures can be identified (see Figs. 1.16 and 1.18): the ovarian stroma, antral follicles, CLs and ovarian blood vessels. In addition, pathological structures such as ovarian

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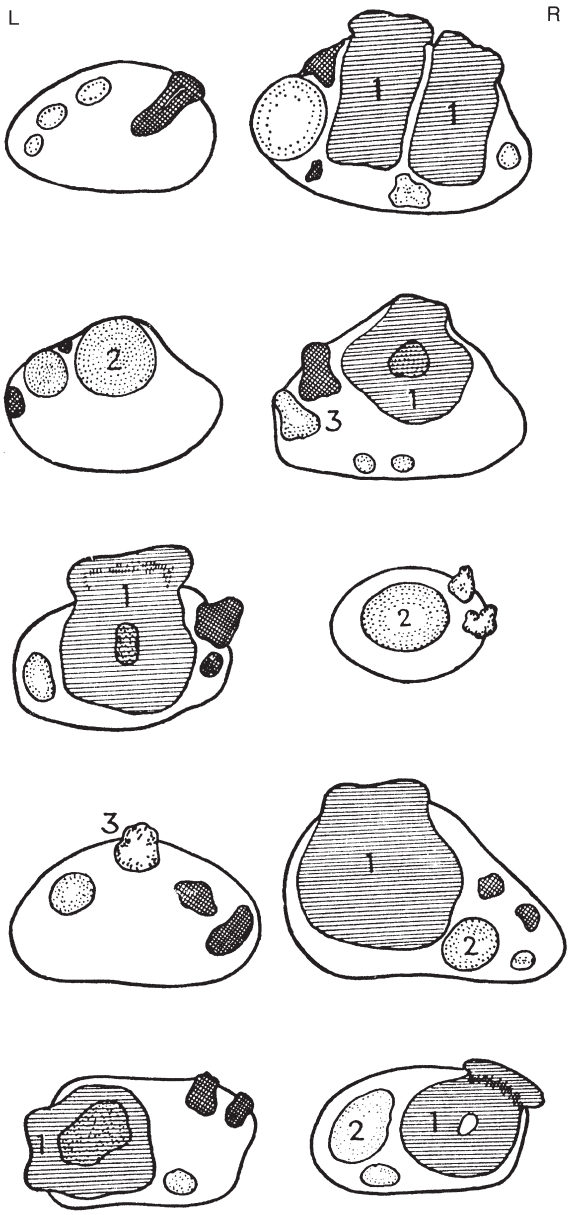
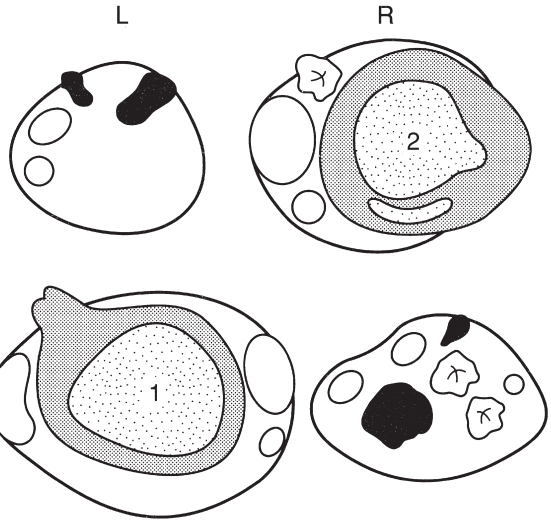


• **Fig. 1.27** Ovaries of parous cows in dioestrus. *(1)* Corpus luteum; *(2)* largest follicle; *(3)* corpus albicans. Stage E in Fig. 1.17.

cysts can be seen; the ovarian stroma has a mottled echotexture. The antral follicles are readily identifiable as anechoic (black) structures of variable size, with a clearly defined line of demarcation between the follicular wall and antrum. Follicles will not always be regular and spherical in shape; typically, follicles with an irregular shape will be atretic. CLs have a well-defined border and a mottled echogenic appearance that is less echogenic than the ovarian stroma. The presence of a fluid-filled lacuna can be readily identified as a dark, nonechogenic area in the centre. Differentiation between developing CLs and CLs that have started to regress can be difficult. As the CL further regresses, it will become isoechogenic with the ovarian stroma (DesCôteaux et al. 2009). Ovarian blood vessels, which can be confused with antral follicles, are black, nonechogenic structures; movement of the transducer will usually demonstrate their elongated appearance.

Over recent years there have been major advances in the use of colour Doppler ultrasound techniques to show haemodynamic



• **Fig. 1.28** Examples of vacuolated bovine corpora lutea, showing single *(1)* and sometimes multiple *(2)* cavities.

changes in the local circulation of the follicle and/or CL. To date, its use has principally been limited to researchers, but the technology offers exciting opportunities. For example, it can be used to measure follicular blood flow enabling identification of normal developing follicle. It can also provide an enhanced imaging modality to distinguish between functional and nonfunctional corpora lutea, i.e., whether the CL is developing or regressing (Bollwein et al. 2016).

Endocrine Changes During the Oestrous Cycle

The trends in concentrations of reproductive hormones in the peripheral circulation are illustrated schematically in Fig. 1.29. It is important to stress that many hormones are secreted in a pulsatile manner and fluctuate considerably day by day; readers are invited to read the descriptions by Peters (1985) and Forde et al. (2011).

Just before the onset of behavioural oestrus, there is a sudden rise in plasma oestrogen, particularly oestradiol. The maturing preovulatory follicle is the source of this oestradiol. Peak values occur at the beginning of oestrus, with a subsequent decline to basal concentrations by the time of ovulation. The prooestrus rise in oestradiol stimulates the LH surge that is necessary for final follicular maturation, ovulation and CL formation; a secondary, less discrete, peak has been demonstrated 24 hours after the ovulatory surge of gonadotrophin (Dobson 1978). During the rest of the cycle, there are fluctuations in plasma oestradiol concentrations along the baseline, although there is a discrete peak around day 6 of the cycle (Glencross et al. 1973). It is hypothesised that this is related to the first wave of follicular growth (Ireland & Roche 1983).

The changes in progesterone concentrations mimic closely the physical changes of the CL. In a number of cows there is evidence of a delay in progesterone production or secretion by the CL (Lamming et al. 1979), which appears to affect the fertility of the individual adversely. Peak values are reached by days 7 and 8 after ovulation and decline fairly quickly from day 18. When progesterone values fall to fairly low basal levels, the removal of the anterior pituitary block allows the sudden release of gonadotrophins. Meaningful prolactin values are frequently difficult to obtain because stress induced by restraint for venepuncture is sufficient to cause a significant rise.

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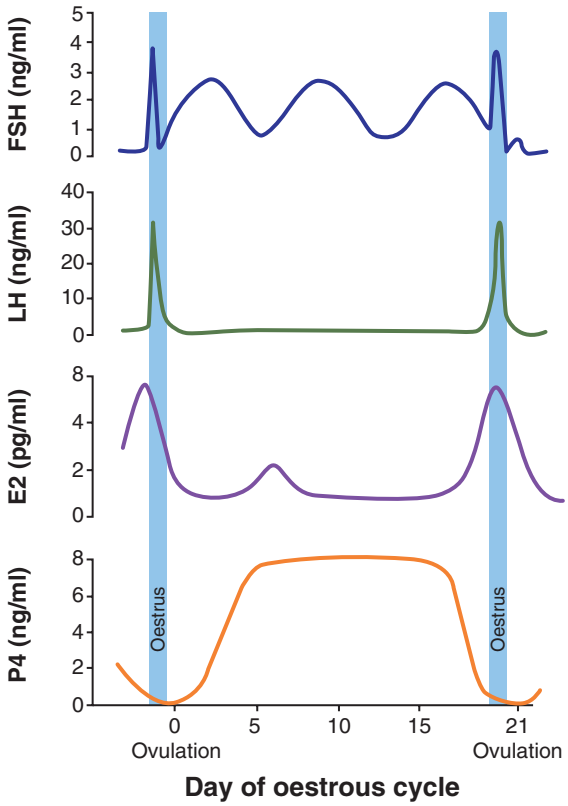

• **Fig. 1.29** Trends in hormone concentrations in the peripheral circulation of the cow during the oestrous cycle, with three follicular waves.

The Sheep

The reproductive season of most breeds of sheep in the UK and Northern Europe is from October to February (it will be the reverse in the southern hemisphere), during which time there are 8 to 10 recurrent oestrous cycles. The stimulus for the annual onset of sexual activity is declining length of daylight. The extent of the breeding season diminishes with increase in latitude; thus at the equator ewes may breed at any time of year, whereas in regions of high latitude – in both northern and southern hemispheres – the breeding season is restricted and distinct, with a prolonged phase of anoestrus after parturition.

The average duration of oestrus in mature ewes of British breeds is about 30 hours and is at least 10 hours less in immature ewes. In Merinos, oestrus may last 48 hours. Ovulation occurs towards the end of oestrus, and the length of the oestrous cycle averages 17 days; it is remarkably consistent between cycles (Bartlewski et al. 2011).

Signs of Oestrus and Mating Behaviour

Oestrous ewes are restless, will seek the ram and together form a following harem. The ram 'tries' members of this group for receptivity, by pawing with a forefoot, rubbing his head along the ewe's side and nipping her wool. A nonreceptive ewe moves away, but when in full oestrus she stands, waggles her tail and moves it laterally. The vulva is slightly swollen and congested, and there is often a slight discharge of clear mucus. The ram mounts and makes a series of probing pelvic thrusts and then dismounts. After variable intervals further mounts occur before intromission is achieved, characterised by a deep pelvic thrust. An essential feature of successful coitus in the Najdi and Awassi breeds of the Middle East is the lateral displacement by the ram of the fat tail of the ewe. Rams of other breeds are unable to move the tail sufficiently to perform intromission.

More ovine matings occur during early morning and evening. When several rams run with a flock, a hierarchy is established, and the dominant male attracts the largest harem. Despite this, a majority of ewes will mate with more than one ram. In addition, ewes show a preference for rams of their own breed or for a ram of their particular group if that group is mixed with other groups of different origin (Lees & Weatherhead 1970). The number of services received by an oestrous ewe averages a little above four. Rams may serve between 8 and 38 ewes in a day.

Changes in the Ovaries

The ovaries of the ewe are smaller than those of the cow, and their shape is nearer the spherical. During anoestrus their size is approximately $1.3 \times 1.1 \times 0.8$ cm, and the largest follicles present vary from 2 to 6 mm. The time period from the primordial to preovulatory follicular stage exceeds 6 months in ewes. Studies of follicular waves are more difficult in ewes than in the cow or mare because transrectal, ultrasonic, sequential access to the ovaries is much more difficult. Although there is some conflicting evidence, most studies indicate that there are two to four follicular waves per oestrous cycle in ewes (Evans 2003). If there are three waves, then there are two during the luteal phase, with the final and ovulatory wave occurring during the follicular phase (Noel et al. 1993, Bartlewski et al. 2011). There is less certainty about the exact endocrine mechanisms involved in folliculogenesis. However, it appears that the stimulus for the emergence of the follicular wave is the transient increase in FSH and that subsequent growth and demise are independent of FSH (Bartlewski et al. 2011). It is also likely that the IGF system, described in the cow, is involved in the selection of the dominant follicles (Scaramuzzi et al. 2011). Because ewes frequently have double ovulations, or more in prolific breeds, the follicles that ovulate can arise from either the same follicular wave or from the subsequent one. This suggests that the follicular dominance is weaker in sheep than in monoovular species (Bartlewski et al. 1999a, Gibbons et al. 1999). Even during anoestrus, folliculogenesis occurs with follicles reaching the size of those frequently present during cyclical activity (Ravindra & Rawlings 1997, Bartlewski et al. 1998).

Follicles destined for ovulation are 6 to 7 mm in diameter, with smaller subordinate anovulatory follicles (5–7 mm). The follicle walls are thin and transparent, and the follicular fluid when viewed externally appears purple in colour. Ovulation of the follicle is preceded by the elevation of a small papilla that develops above the ovarian surface; then ovulation occurs through rupture of this papilla about 36 hours after the onset of oestrus (Cardwell et al. 1998). The development of the CL is rapid, being linear from day 2 to 12 after ovulation (Jablonka-Shariff et al. 1993, Duggavathi et al. 2003). By day 5 of cycle, it is 6 to 8 mm in diameter and attains its maximum size of 11 to 14 mm (Bartlewski et al. 2011). The prolific breeds tend to have smaller ovulatory follicles, and the resulting CLs tend to be smaller (Bartlewski et al. 2011). As the CL develops, its colour changes from blood red to pale pink. Its size remains constant once it has reached its maximal size until the onset of the next oestrus (Bartlewski et al. 1999b). Then luteal regression is rapid and the colour changes, first to yellow and later to brownish yellow.

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The luteolytic mechanism is similar to that in the cow: at the end of the luteal phase, there is an increase in the number of uterine oxytocin receptors, and, at the same time, luteal oxytocin stimulates uterine $PGF_{2\alpha}$ secretion and vice versa. The upregulation of the oxytocin receptors is principally regulated by oestradiol and progesterone (Mann et al. 1999). The first CLs formed after the first ovulations at the beginning of the breeding season have a shorter life span than subsequent ones. In twin ovulations, the CLs may occupy the same or opposite ovaries. During pregnancy the CL is a similar size (10–14 mm in diameter) as the cyclic CL (Schrick & Inskeep 1993). Its colour is pale pink, but the central cavity has disappeared, having become filled by white, fibrous tissue.

Ovulation with CL formation, but without signs of oestrus, may occur towards the end of anoestrus and certainly at the first ovulation of the new breeding season. As to the number of oocytes shed at oestrus, genetic and nutritional factors play a part. Hill sheep in the UK generally have one lamb, but if they are transferred to lowland pastures where pasture is rich (before the onset of the breeding season) twins become common. With lowland breeds, the general expectancy is an average of 1.5 lambs per ewe. Age is also a factor in the incidence of twinning, with primiparous ewes very much less likely to have twins than multiparous ones. The maximum prolificacy occurs when ewes are 5 to 6 years old, after which it remains constant. Ewes of the Border Leicester and Lleyn breeds are the most prolific of British sheep and commonly bear triplets. The Finnish Landrace and Cambridge breeds normally produce 2 to 4 lambs per pregnancy. Precise genetic mutations have been identified that affect ovulation rates in high prolificacy breeds such as Inverdale, Hanna, and Booroola (McNatty et al. 2002).

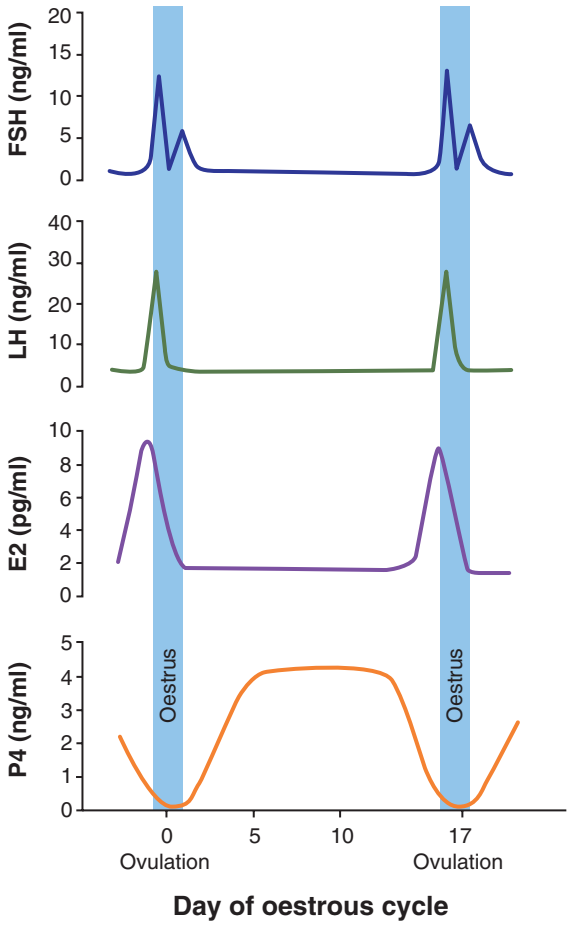
Endocrine Changes During the Oestrous Cycle

The endocrine changes are shown in Fig. 1.30. Just before the onset of oestrus, there is an increase in oestrogens in the peripheral circulation, particularly 17β-oestradiol. This is followed by a sudden surge of LH, which reaches a peak about 14 hours before ovulation and, coincidental with this peak, is a rise in FSH. There is also a second FSH peak 2 days after ovulation (Baird 1978, Rawlings & Cook 1993).

Progesterone concentrations follow closely the physical changes of the corpora lutea, but maximum values are lower than those of the cow, i.e., 2.5 to 4 ng/ml (Bartlewski et al. 2011). Prolactin fluctuates throughout the oestrous cycle; however, concentrations rise during oestrus and ovulation, presumably reflecting the role of this hormone in the formation of the CLs (Campbell et al. 1990).

The Goat

The breeding season in the Northern hemisphere is from August to February, with the greatest activity in the months of October, November, and December. Near the equator there is no evidence of seasonality but continuous cyclic activity. The doe (nanny) goat is polyoestrous, with an average interoestrous interval of 21 days, although this is highly variable particularly at the beginning of the breeding season. The duration of oestrus is 24 to 48 hours (mean 36 hours) and is dependent on age, breed and presence of the male. For example, Angora goats have short oestrus (about 24 hours), whereas Boer goats have relatively long oestrus (about 37 hours). The time from onset to LH surge varies between breeds and individuals, but is typically in the first part of oestrus (approximately 12 hours after oestrus onset) with ovulation occurring 20 to 26 hours later. This means that ovulation relative to oestrus occurs 2 to 36 hours after the onset (Fatet et al. 2011) and generally to the later stages of oestrus.



• **Fig. 1.30** Trends in hormone concentrations in the peripheral circulation of the ewe during the oestrous cycle.

Oestrous behaviour has two phases: (1) proreceptivity in which the doe seeks out and stimulates the male; and (2) receptivity in which the doe will express an immobilisation reflex to the male that involves nudges and standing for mating (Fatet et al. 2011). The detection of oestrus in a doe is difficult in the absence of a male goat. The vulva shows some evidence of oedema and hyperaemia and the tail will wag from side to side and up and down (the most reliable sign according to Llewelyn et al. (1993)). The doe is also restless and more vocal, has a reduced appetite and milk yield and shows mounting behaviour. The presence of the pheromones from the male goat, which can be transferred from the scent gland on to a cloth, will often intensify the signs.

The ovaries are of variable shape, depending upon the structures that are present; the longest dimension is about 2.2 cm. Ultrasonographic studies revealed each cycle has two to six follicular waves, although three to four are most prevalent (see review by Evans (2003)). The preovulatory follicles are 6 to 9 mm in diameter and, when they protrude from the surface, often have a bluish tinge. When multiple ovulations occur, the follicles involved usually arise from the same wave; however, in some cases they arise from consecutive waves. The CLs can be detected 3 days after ovulation and reach 12 mm in diameter by day 8 (Medan et al. 2003). They are pink in colour.

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The endocrine changes measured in the peripheral circulation are very similar to those of the ewe (Medan et al. 2003, Fatet et al. 2011).

The Pig

The domestic sow is generally considered to be polyoestrous, but in the wild she is a seasonal breeder, with the main period of activity being late autumn, and a second peak in April (Claus & Weiler 1985). There is some evidence of a photoperiodic influence on reproduction in the domestic sow, with anoestrus more prevalent in summer and less so in February and March. Equally, the ovulation rate is lower in the summer. For the most part the recurrent reproductive cycles of 21 days are interrupted by pregnancy and lactation. Oestrus is typically 40 to 60 hours in duration (mean 53 hours), with spontaneous ovulation occurring between 38 and 42 hours from the beginning (Signoret 1971); oestrus tend to be shorter in gilts. During lactation the physical stimuli of suckling suppresses cyclical activity, but many sows show an anovulatory oestrus 2 days after farrowing. When weaning occurs at 5 or 6 weeks, oestrus can be expected in 4 to 6 days; earlier weaning results in a slightly longer time interval (Soede et al. 2011).

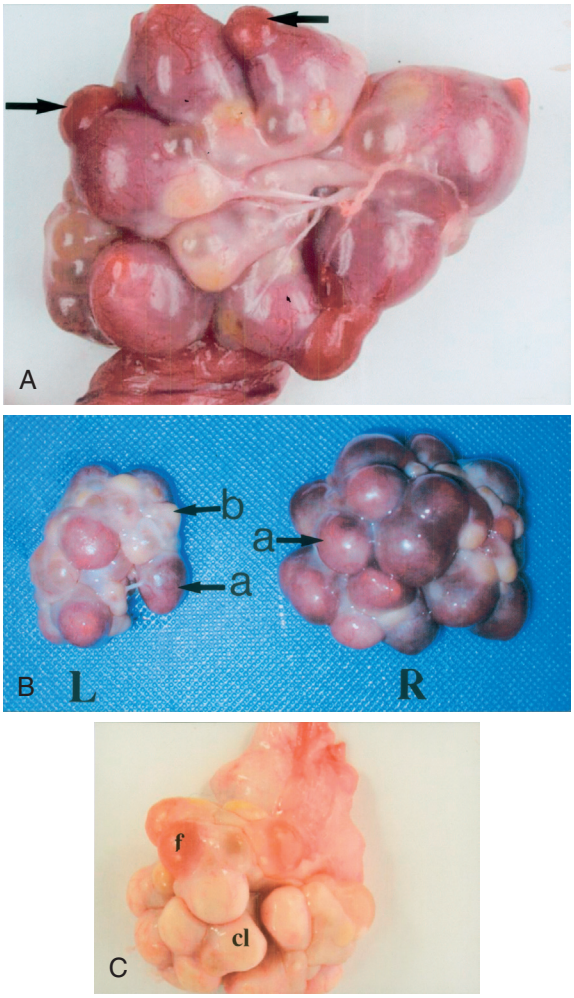
Signs of Oestrus

Beginning 3 days before oestrus, the vulva becomes progressively swollen and congested; these features persist throughout oestrus and then gradually subside during the 3 days afterwards. Restlessness is an unfailing sign of the approach of oestrus, and a peculiar repetitive grunt is emitted. With other sows, the prooestrous animal sniffs their vulvae and may try to ride them or will be the recipient of such attentions. The boar is sought, and in his presence the prooestrous female noses his testes and flanks and may mount him, but will refuse to be mounted. At the height of oestrus, the sow assumes a stationary, rigid attitude with her ears cocked, and she appears to be quite oblivious to her environment. The olfactory and tactile stimuli from the boar are important to observe a standing response. The sow generally remains still during coitus, which lasts an average of 5 to 7 minutes; however, when mating with heavy boars, gilts may become fidgety. Oestrus can be readily detected by firmly pressing the sow's loins with the palms of both hands, such that the oestrous sow will stand motionless with cocked ears. This is known as the back pressure test; the sow not in oestrus will object to this approach (Signoret 1971).

The same immobilisation response can be elicited if the attendant sits astride the sow, and it can also be obtained in the absence of a boar by reproducing the voice or odour of the boar. The substance responsible for boar odour has been identified as 5α-androst-16-ene-3-one and is secreted by the salivary glands (Patterson 1968). In the form of an aerosol, it can be sprayed in the vicinity of sows to promote the standing reaction of oestrus. Silent heat occurs in about 2% of porcine cycles.

Cyclic Changes in the Ovaries

The ovaries of the mature cyclical sow are relatively large and mulberry-like (Fig. 1.31); the lobulated appearance is due to the presence of many large follicles and CLs. The mature, preovulatory follicles attain diameters of 7 to 9 mm. It is much more difficult to study follicular dynamics in the sow than in the cow and mare. First, there are the problems of identifying an individual follicle among large numbers of similarly sized follicles; and second, until the development of small transrectal B-mode ultrasound transducer probes, it has been almost impossible to study changes sequentially in the same animal.



• **Fig. 1.31** Ovaries of sows. (A) Ovary of a sow a few days after ovulation showing a large number of reddish purple corpora lutea (CLs), in some of which the ovulation papilla can be seen *(arrow),* giving them a conical shape. (B) Left *(L)* and right *(R)* ovaries of a sow in middioestrus on the left ovary 5 and on the right 14 CLs of the current oestrous cycle can be seen *(arrow a),* and on the left ovary 4 and on the right 5 CLs of the previous cycle can be seen, which are a white/cream colour *(arrow b)*. (C) Ovary from a sow at 15 to 18 days of the oestrous cycle showing the typical, creamy yellow coloured CLs *(cl)* several follicles *(f)* can be seen. (Courtesy Dr. M. Nevel.)

Unlike other domestic species, there are no clear follicular waves whereby follicles develop up to a dominant stage and then regress if not ovulated. It has been shown that, except during the follicular phase of the cycle, there is continuous proliferation and atresia of follicles, so that there is generally a pool of about 30 to 50 between 2 and 7 mm in diameter (Guthrie & Cooper 1996). In the sow any change in plasma FSH concentration is more subtle than in other species; however, more recent evidence indicates that, during the oestrous cycle, there are FSH surges lasting 2 to 3 days that coincide with changes in inhibin and follicle diameter (Noguchi et al. 2010). The absence of a follicular wave pattern may be due to the different hormones produced by the CLs such as oestradiol and inhibin, which exert a negative feedback effect on FSH

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secretion (Driancourt 2001). Between about day 14 and 16 of the cycle, there is a coordinated recruitment of follicles, probably under the influence of gonadotrophin stimulation induced by the decline in progesterone and the withdrawal of its negative feedback effect (Soede et al. 2011). FSH increases the number of recruited follicles, whereas LH is required for growth to preovulatory size. A substantial number of these follicles are destined for ovulation, although it is not possible to identify the preovulatory population until day 21 to 22 of the oestrous cycle. The growth of selected preovulatory follicles during the follicular phase is associated with rapid atresia of small follicles and a block to their replacement in the proliferating pool. This indicates some intraovarian control mechanism (Foxcroft & Hunter 1985). The precise nature of the substances is not fully understood, although various substances have been proposed: steroids, growth factors (epidermal/transforming, fibroblast, and insulin-like), and their related binding proteins (Hunter & Picton 1999).

During lactation LH concentrations and pulsatility are suppressed due to suckling, which inhibits the GnRH pulse generator. As lactation progresses, the suppression of LH wanes, and by the end of lactation sows appear to have synchronised follicular development with follicles reaching approximately 4 to 5 mm in size and then regressing (Soede et al. 2011). At weaning the largest antral follicles will be recruited, and the sow will enter the follicular phase, which lasts 4 to 6 days. Primiparous sows tend to have smaller follicles at weaning than multiparous one. This is likely to result from primiparous sows having a greater negative energy balance during the preceding lactation. As a consequence, primiparous sows have a slightly longer weaning to ovulation interval (WOI) (Soede et al. 2011).

The preovulatory follicle is pale pink in colour with a fine network of surface blood vessels and a very transparent focus that indicates the site of imminent ovulation. The pool of preovulatory follicles is not homogenous, with both larger and smaller follicles present. The LH surge will trigger ovulation and luteinisation of the preovulatory follicle, with ovulation occurring approximately 30 hours after the LH peak. Ovulation itself is likely to occur over a 1- to 3-hour time period; haemorrhagic follicles are common. After ovulation there remain a considerable number of follicles of about 4 mm, which will regress thereafter.

Typically between 15 and 30 follicles will be ovulated each oestrous cycle. Ovulation rate is influenced by breed and factors that affect the growth and development of antral follicles (e.g., nutrition). Thus sows with a more severe negative energy balance are likely to have a reduced ovulation rate.

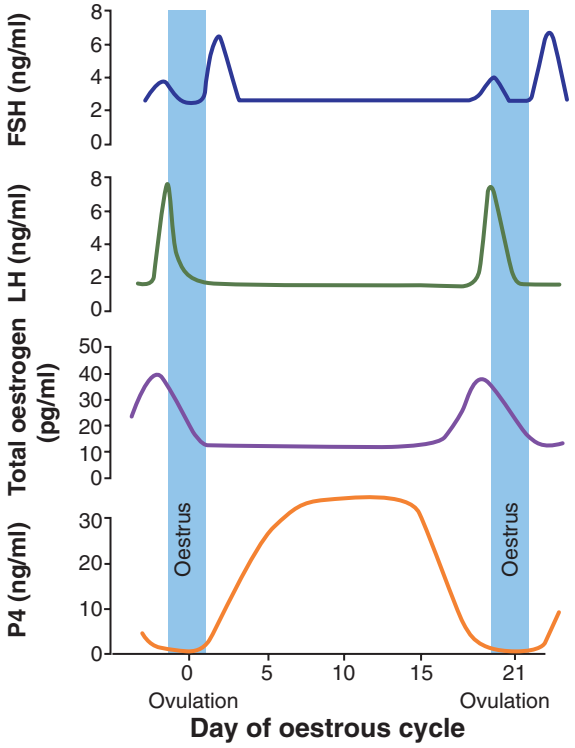
Immediately after ovulation, the ruptured follicle is represented by a congested depression, but the accumulation of a blood clot soon gives it a conical shape (Fig. 1.31A). By day 3, its cavity is filled with a dark red blood clot, which by day 6 is replaced by a connective tissue plug, or yellow fluid (probably serum); this may persist up to day 12. The CLs attain their maximum size at 7 to 9 days, after which they gradually regress to the next oestrus. They are a dark red colour up to day 3, but then change to and remain a red wine colour until day 15 (Fig. 1.31B). As the CLs regress between days 15 and 18, the colour changes rapidly from red wine to yellow, creamy yellow or buff (Fig. 1.31C).

The mechanism of luteolysis is not fully understood in the sow. Although $PGF_{2\alpha}$ is secreted by the uterus as early as days 12 to 16 of the cycle, which is earlier than in other species (Weems et al. 2006), the role of oxytocin in stimulating $PGF_{2\alpha}$ release remains unresolved. The CLs are unresponsive to exogenous $PGF_{2\alpha}$ until about 12 days after ovulation; thus prostaglandins cannot be used to synchronise oestrus in pigs. During the process of luteolysis, the CLs are invaded by macrophages that produce tumour necrosis factor (TNF), and this, in combinations with $PGF_{2\alpha}$, probably causes luteolysis (Weems et al. 2006). In addition, there is also a suggestion that TNF inhibits oestradiol production, thereby removing a luteotrophic source.

There is further rapid luteal regression at the next oestrus, but throughout the succeeding dioestrus, the CLs will remain as distinct entities after which they regress rapidly to grey, pinhead foci. Therefore when a cyclic sow is slaughtered in the first half of the luteal phase, the ovaries may show the red wine–coloured CLs of the current cycle along with the smaller, pale CLs of the previous cycle and the grey specks of the third generation CLs (Fig. 1.31B). During the luteal phases of the cycle, oestrogens are luteotrophic; as a result, it is possible to prolong the life span of the CLs for several weeks, resulting in a pseudopregnancy (Conley & Ford 1989).

Endocrine Changes During the Oestrous Cycle

The endocrine changes are shown in Fig. 1.32. Oestrogens in the peripheral circulation start to rise rapidly when the CLs begin to regress, reaching a peak at the onset of oestrus, approximately 36 hours before ovulation (Noguchi et al. 2010). The ovulatory LH peak occurs 8 to 15 hours after the oestrogen peak. For the remainder of the cycle, LH concentrations fluctuate at low levels, whereas FSH concentrations vary throughout the cycle and appear to have some pattern of secretion; thus there are two surges, one concurrent with the LH peak and a larger one on day 2 or 3 of the cycle (Noguchi et al. 2010). The latter peak coincided with the minimum value of oestradiol (Van de Wiel et al. 1981), and as with other species, the progesterone concentrations closely follow



• **Fig. 1.32** Trends in hormone concentrations in the peripheral circulation of the sow during the oestrous cycle.

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the physical changes of the CLs. Peripheral progesterone concentrations reach a maximum of 20 ng/ml, and for the first 8 days after ovulation, there is a good correlation between progesterone levels and the number of CLs; however, by 12 days it is less obvious.

There is a sharp decline in prolactin concentrations within hours of weaning (Shaw & Foxcroft 1985). During the follicular phase two prolactin surges have been identified: the first one is concomitant with the preovulatory LH and oestrogen surges, and a second is just before ovulation (Van de Wiel et al. 1981).

The Dog

Reproductive activity in the bitch differs from the polyoestrous pattern of other species, in that there are no frequent, recurring periods of oestrus, either repeatedly throughout the year as in cattle and pigs or seasonal as in horses, sheep, goats and cats. All bitches have a prolonged period of anoestrus or sexual quiescence between successive oestruses irrespective of whether they have been pregnant or not; this pattern has been described as mono-oestrus (Jochle & Andersen 1977). There is no luteolytic mechanism, and in the nonpregnant bitch the prolonged luteal phase will lasts for 55 to 75 days. It is only comparatively recently that the mechanisms involved in determining the duration of anoestrus and when bitches come into oestrus have started to unfold. In addition, it must be stressed that this, as with much of the work on canine reproduction, has been done on experimental Beagle colonies, which may or may not be representative of the species in general. Anoestrus is 'obligate' enabling time for the endometrium to repair and lasts a minimum of 7 weeks, but averages 18 to 20 weeks (Concannon 2011). Towards the end of anoestrus GnRH pulse frequency and amplitude increases; consequently, FSH concentrations increase in the latter stages of anoestrus. There is strong evidence that dopaminergic substances are involved in initiating folliculogenesis, thus causing the transition from anoestrus to prooestrus and oestrus. When treating bitches with dopamine agonists such as bromocriptine and cabergoline that can shorten the duration of anoestrus, it is possibly acting by inhibiting prolactin secretion; however, metergoline, which lowers prolactin levels via the serotonin pathway, did not shorten the duration of anoestrus. There is evidence that endogenous dopaminergic substances have a direct effect on regulating GnRH secretion, thereby stimulating basal FSH secretion and initiating antral follicle development (see review by Okkens & Kooistra (2006)). Just prior to prooestrus, there is increased LH pulsatility.

The average interval between successive oestrous periods is 7 months, but it is variable (typically 6–10 months), and there is some evidence that the breed of the bitch can have an effect. For example, in the Rough Collie it is 8.5 months (37 weeks) compared to 6 months (26 weeks) in the German Shepherd (Christie & Bell 1971); the other breeds that were studied had mean intervals between these two figures. Mating does not appear to influence the interval, although pregnancy caused a slight increase (Christie & Bell 1971). There does not appear to be any seasonal effect on reproductive function because there is a fairly even distribution of the occurrence of oestrus throughout the year (Sokolowski et al. 1977).

The oestrous cycle is traditionally divided into four phases (Heape 1900).

Prooestrus

The bitch has a true prooestrus characterised by the presence of vulvar oedema, swelling and a sanguineous discharge. Some fastidious bitches show no evidence of discharge, as they are continually cleaning the perineum. The bitch is attractive to males but will not accept the male.

Oestrus

The bitch will accept the male and adopts the breeding stance. The vulva becomes less oedematous, and the vulvar discharge becomes clearer, less sanguineous and less copious.

The duration of prooestrus and oestrus combined is about 18 days, i.e., 9 days each. However, this can be very variable; some bitches showing very little sign of prooestrus before they will accept and stand for the dog, whereas others produce a copious sanguineous discharge during true oestrus. Some bitches also show evidence of sire preference, which can affect the timing. Ovulation usually occurs 1 to 2 days after the onset of oestrus, although, using laparoscopy, it has been observed that some follicles continue to ovulate up to 14 days later.

Metoestrus

This stage starts when the bitch ceases to accept the dog; however, there is dispute about its duration. Some consider that it ends when the CLs have regressed at 70 to 80 days, whereas others measure it in relation to the time taken for repair of the endometrium, namely 130 to 140 days.

Anoestrus

At the end of metoestrus the bitch passes into a period of anoestrus without any external signs; the same is also true after parturition following a normal pregnancy. This phase lasts 2 to 10 months before the bitch returns to prooestrus.

Signs of Oestrus

The first indication that prooestrus is approaching is the onset of slight swelling of the vulvar lips; this generally precedes the commencement of bleeding by several days. Labial swelling is progressive during the prooestrus period. Bleeding attains its maximum early in prooestrus and continues at this level into the early part of true oestrus. During the greater part of prooestrus the bitch, although attractive to the dog, takes no interest in his attentions. She will not stand for him and generally attacks him if he attempts to mount her. A day or so before the end of prooestrus, her attitude changes, and she shows signs of courtship towards the male. These comprise sudden darting movements, which end in a crouching attitude with her limbs tense and her face alert. She barks invitingly, but as the dog approaches she moves suddenly again; she will not yet allow him to mount. With the onset of oestrus, the invitation to coitus is obvious. She stands in the mating position with her tail slightly raised or held to one side, and remains still while the male mounts and copulates. In the later stages of the copulatory tie, which occupies 15 to 25 minutes, she becomes restless and irritable, and her attempt to free herself may cause the male considerable physical embarrassment. After the first 2 days of oestrus, sexual desire gradually recedes but, with the continued persuasion of the male, she will accept coitus until the end of the period. Bleeding, although reduced in amount, generally continues well into oestrus; more often, however, the discharge becomes yellow as oestrus proceeds. Vulvar swelling and tumefaction are greatest at the onset of the stage of acceptance. During the course of oestrus the enlarged labia become softer in consistency; some labial swelling continues into the first part of the metoestrous phase.

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Changes in the Vagina and Uterus

Vaginal Smear

The cyclical changes that occur in vaginal cytology have been described in detail (Griffiths & Amoroso 1939, Schutte 1967, Hewitt & England 2000). Vaginal smears stained with either a simple stain such as Leishman's or a Trichrome stain such as Shorra's can, with practice, be used to determine the stage of the oestrous cycle; the advent of the Diff-Quik staining method has greatly simplified the procedure. At the onset of prooestrus large numbers of erythrocytes are present; however, when true oestrus occurs, the number of erythrocytes is reduced, and the smear consists of superficial cell types from the stratified squamous epithelium such as anuclear cells, cells with pyknotic nuclei, and large intermediate cells. Towards the end of oestrus, numbers of polymorphonuclear neutrophil leukocytes appear in the smear. These, together with small intermediate cells, are the dominant cell types during metoestrus. In anoestrus nucleated basal and intermediate cells of the stratified squamous epithelium, together with a few neutrophils, form the characteristic smear.

Fig. 1.33 shows the cyclical changes of the cell types, and Fig. 1.34 shows examples of stained smears.

Vaginal Epithelium

The vaginal epithelium during anoestrus is of the low, columnar, cuboidal type and comprises two or three layers only. During prooestrus the epithelial cells change to the high, squamous, stratified type and persist in this form until the later stages of oestrus. The stratum corneum and the layers immediately beneath it are lost by desquamation during the prooestrous and oestrous periods, leaving a low, squamous structure that becomes converted to columnar epithelium in 1 to 3 weeks after the end of oestrus. During metoestrus (and pregnancy) the epithelium is of a higher columnar type than during anoestrus.

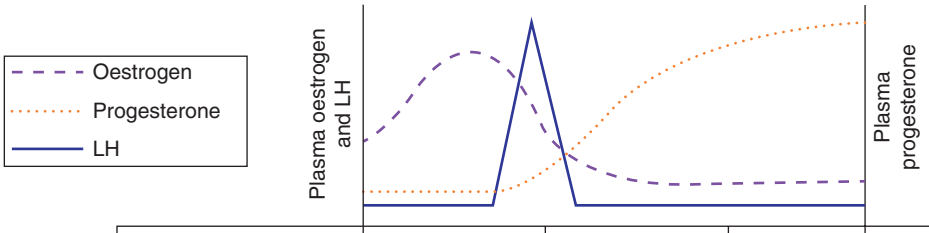
During prooestrus, oestrus and early metoestrus, the epithelium and lamina propria are infiltrated with large numbers of neutrophils, which eventually escape into the vaginal lumen.

Endometrium

The endometrium shows considerable change during the oestrous cycle. The endometrial glands in prooestrus and oestrus are loosely coiled with very obvious lumina and deep epithelial lining. During metoestrus the glands become larger, the lumina smaller, and the coiled parts in the basal layer of the endometrium more tortuous. As the bitch passes into anoestrus, there is a reduction in the amount and degree of coiling of the glands. At about 98 days after the onset of oestrus, i.e., in metoestrus, there is evidence of desquamation of the endometrial epithelium; however, by about 120 to 130 days the epithelium has been restored by proliferation of cells from the crypts of the endometrial glands.

Changes in the Ovaries

During anoestrus the ovaries are oval and slightly flattened. In a bitch of medium size, they measure approximately 1.4 cm from pole to pole and 0.8 cm from the attached to the free border. No



Cell type		Prooestrus Early	Prooestrus Late	Oestrus Early	Oestrus Late	Early metoestrus	Anoestrus	Vaginitis pyometra
Parabasal		+ + +	+	–	–	–	+ +	±
Small intermediate		+ + +	+ +	–	–	+	+	±
Large intermediate		±	+ +	+ + +	+ + +	–	–	±
Anuclear keratinized		–	+ +	+ + + +	+ + +	±	–	–
Red blood		+	+ + +	+ +	–	–	±	±
Neutrophilis		+	–	–	+	+ + +	+	+ + + +

• **Fig. 1.33** Changes in the types of cell and their relative numbers in vaginal smears from the bitch during the different stages of the oestrous cycle, and changes in oestrogen, progesterone and luteinising hormone levels in the peripheral plasma.

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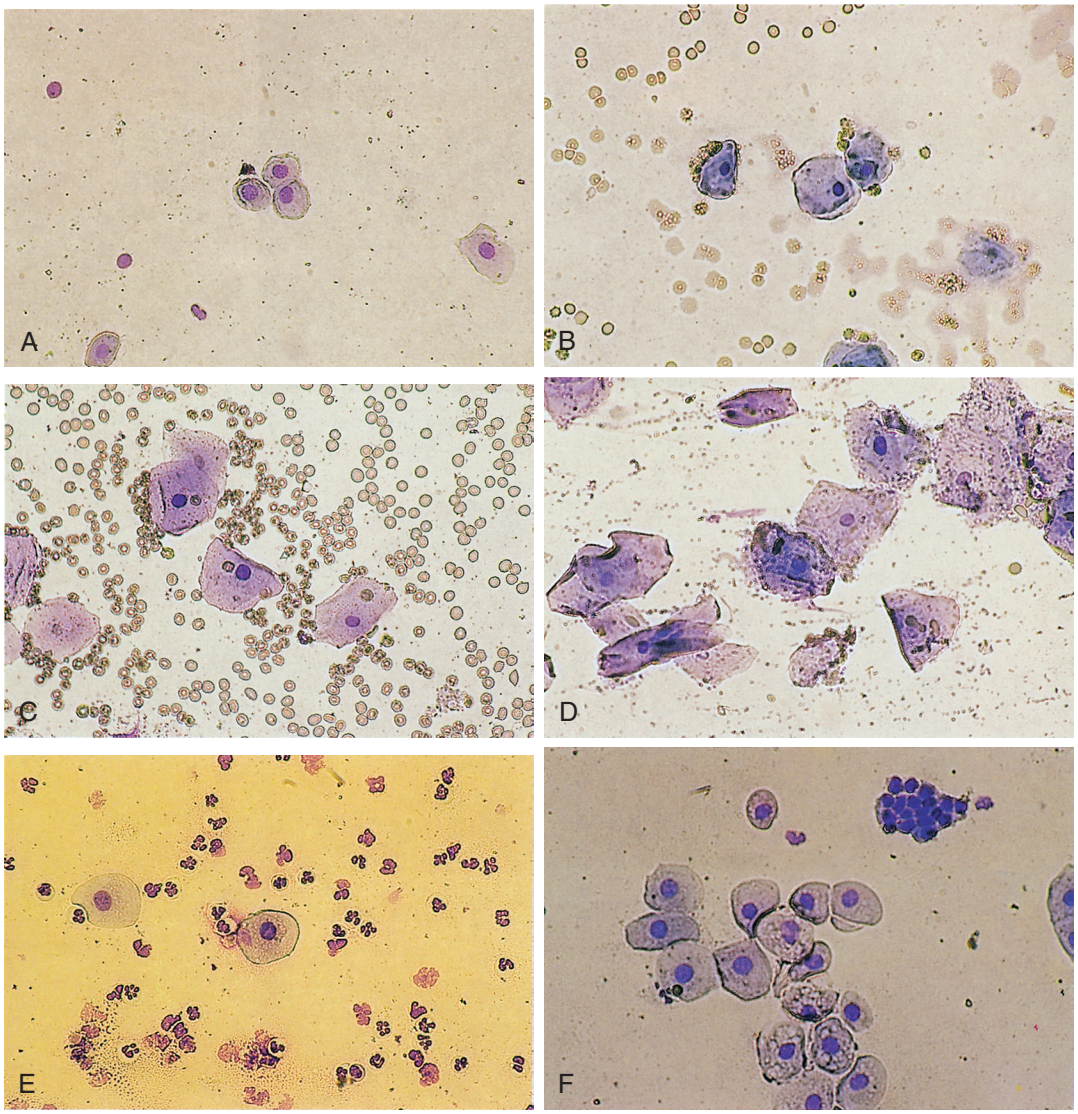

• **Fig. 1.34** Photomicrographs of exfoliative vaginal cells during various stages of the reproductive cycle of the bitch. The smears have been stained with a modified Wright-Giemsa stain. (A) Anoestrus: parabasal epithelial cells and small intermediate epithelial cells. (B) Prooestrus: small intermediate epithelial cells, large intermediate epithelial cells and erythrocytes. Polymorphonuclear leucocytes are also found in low numbers during this stage of the cycle but are not demonstrated here. (C) Early oestrus: large intermediate epithelial cells, anuclear epithelial cells and erythrocytes. Polymorphonuclear leucocytes are generally absent during this stage of the cycle. (D) Oestrus: anuclear epithelial cells, large intermediate epithelial cells and erythrocytes. The percentage of anuclear cells is high. (E) Metoestrus (higher magnification than A = D): small intermediate epithelial cells and large numbers of polymorphonuclear leucocytes. During early metoestrus large intermediate epithelial cells may be present; later numbers of parabasal epithelial cells increase. There is often a large amount of background debris. (F) Late metoestrus (higher magnification than A = D): parabasal epithelial cells and small vacuolated intermediate epithelial cells are typical of this stage of the cycle but may also be found during anoestrus and more rarely during prooestrus.

appreciable follicles can be seen, although on section the minute remnants of the CLs of the previous cycle may be seen as yellow or brown spots. In the young bitch, the surface of the ovaries is smooth and regular, but in the aged animal it is irregular and scarred. At the commencement of prooestrus, developing follicles have already attained a diameter of 4 mm (England & Allen 1989). They progressively enlarge until, at the time of ovulation, their size varies from 7 to 11 mm (England & Allen 1989, Hayer et al. 1993). By this time the ovary is considerably enlarged, its size depending on the number of ovulatory follicles present; its shape is irregular because of the projection of the follicles from its surface. Because of the thickness of the follicle wall, it may be difficult to distinguish between follicles and CLs. Prior to ovulation, the surface of the follicle shows a slightly raised papule, pinhead-sized, and the epithelium covering it is brown, which contrasts with the flesh colour of the remainder of the follicle. A remarkable feature of the ovulatory follicle of the bitch is the thickness of its wall. This is due to hypertrophy and folding of the granulosa cells, which can be seen on section with the naked eye as evidence of preovulatory luteinisation.

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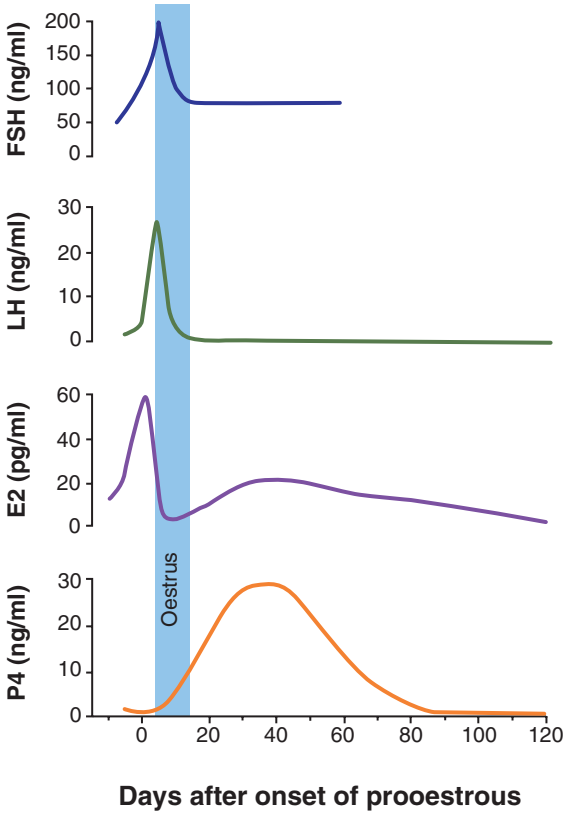
Ovulation is spontaneous, and normally occurs 48 to 60 hours after the LH surge (Concannon 2011). Ovulation is nearly always synchronous among the ovulatory follicles (Concannon 2011). Unlike other domestic species in which a secondary oocyte is ovulated, the oocyte in the bitch is ovulated at the primary stage. The oocyte will then mature and become a secondary oocyte and is capable of being fertilised after 96 to 108 hours. The secondary oocyte remains viable for 24 to 48 hours (day 5 or 6 after LH surge), but occasionally up to 144 hours (day 10) after ovulation (Tsutsui et al. 2009). The CL at first contains a central cavity, but becomes filled by compact luteinised cells within 10 days of ovulation, by which time the body has attained its full size (6–10 mm); CLs now comprise the greater mass of the ovary. As a rule, an approximately equal number of CLs are found in each ovary, although occasionally there are wide differences. In this connection it is interesting to note that the numbers of fetuses in the respective uterine horns during pregnancy frequently differ from those of the CLs in the ovaries on the respective sides. Thus it appears that embryonic migration into the uterine horn on the contralateral would appear to be common. On section the CL is yellowish pink in colour, and it remains unchanged in the nonpregnant bitch until about the 30 days after ovulation, after which it slowly undergoes atrophy. Visible vestiges may be present throughout anoestrus. During pregnancy the CLs persist at their maximum size throughout, but regress fairly rapidly after parturition.

Ultrasound Appearance of the Ovaries

Using a transabdominal approach via the flank with the bitch standing and a 7.5 MHz real-time linear array transducer, England and Allen (1989) were able to identify ovarian structures at the onset of prooestrus that were circular and anechoic and were obviously developing antral follicles. When the bitch was in oestrus, they had increased in size, reaching a maximum of 4 to 10 mm in diameter on day 13 (day 0 being onset of prooestrus) (Hayer et al. 1993). The walls of the follicles became thickened from day 10 onwards, presumably because of preovulatory luteinisation. Ovulation was characterised by decreased number of fluid-filled follicles with similarly-sized hypoechoic structures. There is further gradual thickening of the wall as the CLs develop, with the eventual obliteration of the central anechoic cavity (England & Yeager 1993); there was no evidence of follicular collapse associated with ovulation. At 25 to 30 days after the onset of prooestrus, the ovaries were difficult to identify.

Endocrine Changes During the Oestrous Cycle

The trends in endocrine changes are shown in Fig. 1.35. The main feature that distinguishes them from other domestic species is the prolonged luteal phase, illustrated by the persistence of high progesterone levels in the peripheral blood. It is noticeable that progesterone levels start to rise before ovulation has occurred, which confirms the morphological evidence of preovulatory luteinisation of the mature follicles 60 to 70 hours before ovulation (Concannon 2011). This preovulatory rise in progesterone may provide the stimulus for the bitch to accept the male (Concannon et al. 1975). In addition, it is used as a method to determine the optimal timing of artificial insemination. In that peripheral plasma progesterone concentrations should have increased above the baseline (less than 1 ng/ml), with mating or insemination planned for 4 to 6 days after the progesterone rise is first detected



• **Fig. 1.35** Trends in hormone concentration in the peripheral circulation of the bitch during the oestrous cycle. (From Jeffcoate 1998; de Gier et al. 2006.)

or, ideally, the day after values exceeded a threshold as determined by the assay kit (Hewitt & England 2000).

Oestradiol rises rapidly during prooestrus and peaks just before the onset of standing oestrus; this is accompanied by an increase in androstenedione and testosterone concentrations. An oestradiol peak occurs on average one day before the LH peak (de Gier et al. 2006), which lasts much longer than that of other species (36 ± 55 hours; de Gier et al. (2006)), with ovulation occurring 48 to 60 hours after this (Phemister et al. 1973, Concannon 2011). The preovulatory LH surge is generally accompanied by the FSH surge, with FSH concentrations reaching a peak at oestrus and coinciding with that of LH, but lasting much longer (110 ± 8 hours; de Gier et al. (2006)). Peripheral progesterone concentrations continue to increase after ovulation and peak around 25 days after ovulation. Thereafter, progesterone concentrations will gradually decline and remain above 1.5 ng/ml until day 70 (Concannon 2011).

Prolactin is required in addition to LH to support luteal function in the bitch. Prolactin appears to have a negative correlation with progesterone from about 25 days after ovulation; thus as progesterone levels fall towards the end of metoestrus or pregnancy, prolactin concentrations increase and therefore maintains the CLs. It is the major luteotrophic hormone in this species. During pregnancy there is enhanced secretion of prolactin from the placenta, which is responsible for elevated progesterone concentrations in the pregnant bitch compared with nonpregnant counterparts.

Progesterone and oestradiol concentrations are low during anoestrus. LH is also generally low, but there will sporadic spikes of LH. Conversely, FSH concentrations will be high (presumably due to lack of negative feedback of progesterone and oestradiol on the hypothalamus) with sporadic spikes of FSH that are concomitant

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with the LH spikes. FSH will gradually increase towards prooestrus (Concannon 2011); during prooestrus, FSH concentrations will decrease as the oestradiol concentrations rise.

Pseudopregnancy

Most, if not all, bitches show some evidence of pseudopregnancy during metoestrus, with intensity and signs being very variable. For this reason it is preferable to refer to *covert* pseudopregnancy, in which the bitch will be in metoestrus but will show little or no signs, and *overt* pseudopregnancy. In the latter, the clinical signs will range from slight mammary development and lactogenesis, while at the opposite extreme the bitch will show all the external signs of pregnancy and will ultimately undergo a mock parturition, with nesting, loss of appetite, straining, emotional attachment to inanimate objects and heavy lactation.

Pseudopregnancy was originally believed to be due to an intensification and prolongation of metoestrus; however, a number of studies have demonstrated that there is no difference in the progesterone concentrations in the peripheral blood of bitches with or without signs of pseudopregnancy. It is likely that prolactin is responsible for initiating the changes, with the decline in progesterone appearing to coincide with the rise in prolactin. If bitches undergo ovariohysterectomy when they are pseudopregnant, then the condition can be intensified and prolonged. Furthermore, prolactin inhibitors such as bromocriptine and cabergoline have been successfully used to cause remission of the signs of pseudopregnancy and are now widely used in veterinary clinical practice.

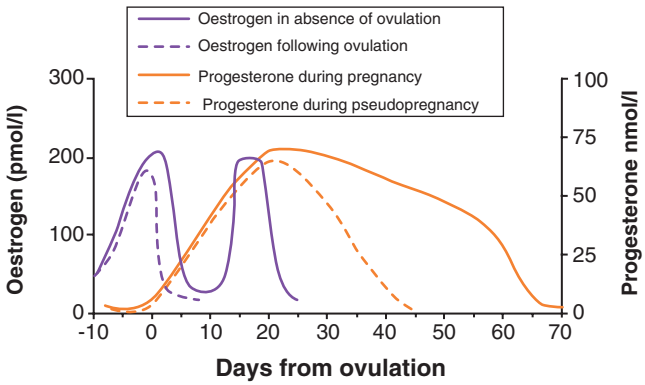
The Cat

In most breeds, females will usually show their first oestrus once a body weight of 2.3 to 2.5 kg has been attained, at an approximate age of 7 months. Puberty is also influenced by the season of birth; females born very early in the year may mature in the autumn of that year, whereas those born later will not normally show oestrus until the following spring (Gruffydd-Jones 1982). The time of onset of puberty is much more variable in pedigree cats (Jemmett & Evans 1977).

Free-living nonpedigree and feral cats are seasonally polyoestrous, with a period of anoestrus beginning in the late autumn. Increasing daylight length is the most important factor inducing the resumption of reproductive activity, and the first oestrus usually occurs after the shortest day of the year. There may be a period of apparent lack of oestrous activity in the early summer, but this corresponds with the pregnancy or lactation following mating earlier in the year rather than true anoestrus. Essentially, there are four possible outcomes following oestrus in the queen:

1. A mating occurs that induces ovulation (24–36 hours later), CL formation and if there is a fertile mating, then pregnancy is likely to occur.
2. A mating occurs; there is likely to be ovulation, CL formation, but no fertilisation and a pseudopregnancy will ensue.
3. Mating may or may not occur; there may be no ovulation, which will be followed by follicular regression and atresia and, after a short interoestrous interval of a few days, a return to oestrus. In some cases, the interoestrous interval is so short that some individuals may appear to be continuously oestrus.
4. At the end of the breeding season, in which there is no ovulation, the queen cat may become anoestrus.

Some nonpedigree cats have regular oestrous cycles lasting approximately 3 weeks, but others may show no regular pattern



• **Fig. 1.36** Changes in the mean peripheral plasma concentrations in the queen cat of progesterone (during pregnancy and pseudopregnancy) and oestrogen (in the absence of ovulation and following ovulation).

(Shille et al. 1979). The duration of oestrus is 5 to 8 days and is not significantly shortened by mating (Shille et al. 1979, Wildt et al. 1981). Oestrous cycle patterns show considerably more variation in pedigree cats. Long-haired cats may have only one or two oestrous cycles each year, whereas the period of oestrus may be longer in Oriental queens with a reduced interoestrous interval. Oestrogen concentrations increase dramatically at the time of oestrus from the baseline of 60 pmol/l and may double within 24 hours, reaching a peak of up to 300 pmol/l (Shille et al. 1979) (Fig. 1.36). The principal oestrogen produced by the ovary is 17β-oestradiol. The rapid rise in oestrogen concentrations corresponds to an abrupt appearance of behavioural changes indicative of oestrus, and queens do not usually show a distinctive prooestrous phase.

The oestrual display is characterised by increased vocalisation, rubbing and rolling. The queen becomes generally more active and will solicit the attention of a tom; she may adopt the mating posture either in response to the tom or, occasionally, spontaneously. She lowers her front quarters with the hind legs extended, resulting in lordosis. The tail is held erect and slightly to one side. There may occasionally be a slight serous vaginal discharge, but there are usually no changes in the appearance of the external genitalia. The extent of the oestrual display varies considerably between queens, but is generally more prominent in the Oriental breeds.

Mating and Ovulation

During mating the tom mounts the queen and grasps her neck with his teeth. The queen's hind legs paddle as he adjusts his position, and this becomes more rapid during coitus, which lasts up to 10 seconds. The queen cries out during copulation, and as the tom dismounts, she may strike out at him, displaying the typical rage reaction. This is followed by a period of frantic rolling and licking at the vulva. As soon as this postcoital reaction has ceased, the tom will usually attempt to mate the queen again, and there may be several matings within the first 30 to 60 minutes.

At oestrus, there are 5 to 8 follicles present in both ovaries, and they are 3 to 4 cm in diameter at the time of ovulation (Wildt et al. 1980, Shille et al. 1983); the cat is an induced ovulator, and thus mating is important in triggering ovulation. Receptors are present within the queen's vulva that are stimulated during copulation, ultimately triggering the release of LH from the anterior pituitary. Only about 50% of queens will ovulate after a single mating, and multiple matings may be required to ensure adequate release of LH to induce ovulation (Concannon et al. 1980). The

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ovulatory surge of LH begins within minutes of coitus, peaks within 2 hours and returns to basal values within about 8 hours; peak LH concentrations of greater than 100 ng/ml have been reported (Shille et al. 1983, Tsutsui & Stabenfeldt 1993). Further matings before the peak of LH concentrations has been reached will lead to additional increments. However, after multiple matings over a period of 4 hours or more, further matings may fail to induce any additional increase in LH concentrations. This is thought to result from depletion of the pituitary pool of the hormone or development of refractoriness to GnRH (Johnson & Gay 1981).

Ovulation is an all or nothing phenomenon, and once significant concentrations of LH have been achieved all ovulatory follicles will rupture (Wildt et al. 1980). It has been estimated that ovulation occurs 30 to 40 hours after coitus (Wildt et al. 1980, Shille et al. 1983). The mean ovulatory rate for nonpedigree cats is approximately four, but is more variable in pedigree animals.

Occasionally, ovulation will occur in the absence of any contact with entire toms. Receptors similar to those found in the vulva are also located in the lumbar area (Rose 1978), and these may be stimulated if the queen is mounted by other females or castrated male cats, or even by stroking over this area. Neutered toms may mate queens in oestrus, even if they have been castrated prepubertally. However, in a study involving a colony of American shorthaired cats in which the queens were housed individually and in which tactile stimulation of the hindquarters and perineal regions by handlers was avoided, ovulation was detected as defined by progesterone concentrations great than 4.8 nmol/l in the peripheral circulation. The queens had sight and sound of other cats, including males. It is noteworthy that six of the seven cats that ovulated were older than 7 years of age (Lawler et al. 1993).

Pseudopregnancy

Sterile matings that successfully induce ovulation will lead to pseudopregnancy. Concentrations of progesterone are very similar to those of pregnancy for the first 3 weeks (Fig. 1.36), after which levels gradually fall, reaching baseline by approximately 7 weeks (Paape et al. 1975, Shille et al. 1979) (Fig. 1.36). Oestrus will usually occur shortly afterwards.

Nesting behaviour and milk production are rarely seen in pseudopregnant queens, but hyperaemia of the nipples will usually be evident as in pregnancy. The queen's appetite may increase, with some redistribution of fat leading to an increase in abdominal size.

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2

Reproductive Physiology of Male Animals

TIMOTHY J. PARKINSON

The reproductive organs (Fig. 2.1) of the male animal have three major functions: production of spermatozoa in the testis, maturation, storage and transport of spermatozoa within the duct system, and deposition of semen within the female genital tract via the penis. The functions of the male hormones are also threefold: maintenance of spermatogenesis, production of masculine behaviour (primarily libido and aggression), and development of the masculine body form.

Anatomy of the Male Reproductive System

Testis, Spermatic Cord, and Scrotum

The testes of all domestic male animals are located at the inguinal region within a scrotum. In the bull and the ram this structure is pendulous and has an elongated neck**,** but in most other domestic species the scrotum is closely applied to the inguinal region. The scrotum consists of a skin pouch overlying various fibroelastic and muscular layers, of which the most prominent is the tunica dartos (Fig. 2.2). The dartos layers are confluent between the testes, where they form the intertesticular septum. In the boar the external spermatic fascia is also prominent. The testis itself is surrounded by two layers of peritoneum, which are formed during its descent into the scrotum, as a single outpouching of the parietal peritoneum, through the inguinal canal. The outer layer of peritoneum, the processus vaginalis (tunica vaginalis reflexa), is reflected on to the testis to form the serous outer layer of that organ, the tunica vaginalis propria. Accompanying this outpouching of peritoneum through the inguinal canal is a diverticulum of the internal abdominal oblique muscle, which inserts on to the cremasteric fascia and the vaginal tunics. This muscle, the cremaster, raises or lowers the testis in response to the immediate environmental temperature or noxious stimuli.

The capsule, or tunica albuginea, of the testis is composed principally of fibrous tissue but has a smooth muscle component whose function is largely unknown. Overlying the capsule is the tunica vaginalis propria. The main blood vessels of the testis are distributed over the surface of the tunica albuginea, before penetrating the capsule to supply the testicular parenchyma; the innervation of the testis is mainly confined to the periphery, and little nervous tissue is found in its substance. The substance of the testis (Fig. 2.3) is composed of two main tissues: seminiferous tubules and interstitial tissue. Each seminiferous tubule is a highly convoluted, unbranched tube that opens at both ends into collecting tubules and then into the rete testis. The seminiferous tubules are limited by a basement membrane, which is partially surrounded by contractile myoid cells. Within the tubule the seminiferous epithelium is composed of two main cell lines: somatic Sertoli cells and the sperm-producing germinal cell lines. Interstitial tissue, which consists of steroid-producing Leydig cells, blood vessels, and lymphatics, exhibits much variation in its quantity and morphology between species. For example, Leydig cells in the ram occur in small clusters around blood vessels, interspersed in relatively large lymphatics, whereas the interstitial tissue of the boar has densely packed Leydig cells and small lymphatics (Fawcett 1973).

At the ends of each seminiferous tubule, there is a transitional zone between spermatogenic tissue and the rete testis. In ungulates and carnivores the rete testis is in the centre of the testis within the fibrous matrix of the mediastinum, although its position differs in rodents and primates. The number of seminiferous tubules opening into each of the transitional zones varies between species, as does the number of channels comprising the rete (Setchell & Breed, 2006). The rete testis consists of a network of interconnecting channels with a simple epithelial lining that moves the sperm into the epididymis. The rete opens into the epididymis via 13 to 20 efferent ducts (Hemeida et al. 1978). These tubules are initially relatively straight but have a long convoluted section before opening into the epididymal duct. Fluid resorption occurs in both rete testis and efferent ducts.

The epididymis is a single, highly convoluted tube into which the vasa efferentia drain the seminiferous tubules. Grossly, the epididymis appears as an approximately cylindrical organ, which is divided into a prominent head that is situated close to the suspension of the testis from the spermatic cord, a smaller, medially situated body, and a distended tail that is continuous with the ductus deferens (vas deferens). The muscular wall of the epididymal duct moves sperm through its lumen by peristalsis so that, during their passage through the epididymis, sperm, which are immature on release from the testis, undergo final maturation. The tail of the epididymis also acts as a reservoir for fully mature sperm, which becomes turgid with the accumulation of stored sperm in sexually active animals. In domestic animals the epididymis is closely applied to the exterior of the testis, whereas in rodents and primates the connection is much looser.

The vas deferens is a relatively thick-walled, muscular tube that acts as both a reservoir for sperm and the means of their conduction between the epididymis and the penis. It is situated mediocaudally within the spermatic cord, in a small diverticulum of peritoneum. In addition to the vas deferens the spermatic cord also contains the arteries, veins, and nerves supplying the testis,

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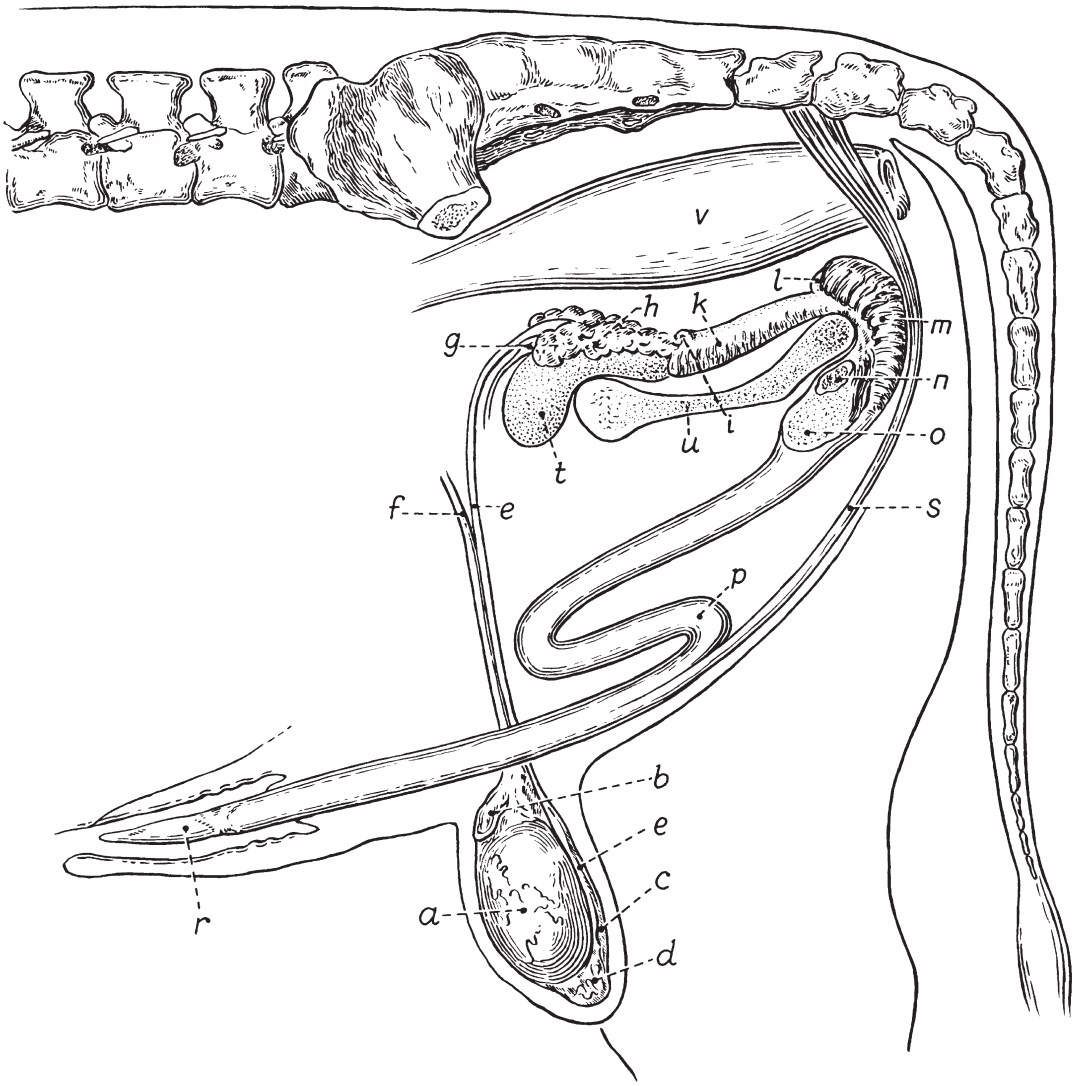
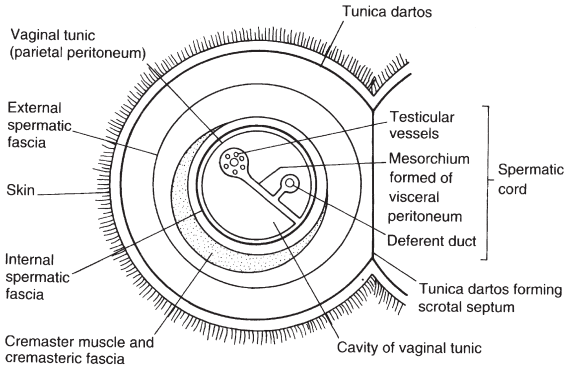


• **Fig. 2.1** The genital organs of the bull: *(a)* testis; *(b)* head of epididymis; *(c)* body of epididymis; *(d)* tail of epididymis; *(e)* vas deferens; *(f)* vascular part of spermatic cord; *(g)* ampulla of vas deferens; *(h)* seminal vesicle; *(i)* body of prostate; *(k)* pelvic urethra surrounded by urethralis muscle; *(l)* bulbourethral gland; *(m)* bulbocavernosus muscle; *(n)* crus penis; *(o)* ischiocavernosus muscle; *(p)* distal sigmoid flexure of penis; *(r)* glans penis; *(s)* retractor penis muscle; *(t)* urinary bladder; *(u)* pubic symphysis; *(v)* rectum. (From Blom & Christensen 1947.)



• **Fig. 2.2** Diagram of the anatomy of the fascial, muscular, and peritoneal tissues of the neck of the scrotum. The vaginal tunic is strongly reinforced by the closely adherent and much thicker internal spermatic fascia. All the layers are closely apposed to their adjacent structures so that the only (potential) space is the cavity of the vaginal tunic. (Redrawn with permission from Cox 1982.)

all of which are contained within the peritoneal vaginal tunics. Together, these structures form the spermatic cord. The spermatic sac includes the spermatic cord, the internal spermatic fascia, cremaster muscle, and cremasteric fascia. The cremaster muscle is situated on the opposite side of the sac to the vas deferens (i.e., on the cranio–lateral surface). The vasa deferentia enter the abdomen through the inguinal canals, from where they run in a caudal direction to join the pelvic urethra where the latter organ joins the neck of the bladder.

A number of short ligaments exist between the various structures within the scrotum, as shown in Fig. 2.4. The proper ligament of the testis joins the ventral pole of the testis to the tail of epididymis, which is also joined to the vaginal tunic by the caudal ligament of the epididymis. These ligaments are derived from the gubernaculum. Finally, on the external surface of the vaginal tunic, the scrotal ligament joins the tunic to the scrotal fascia.

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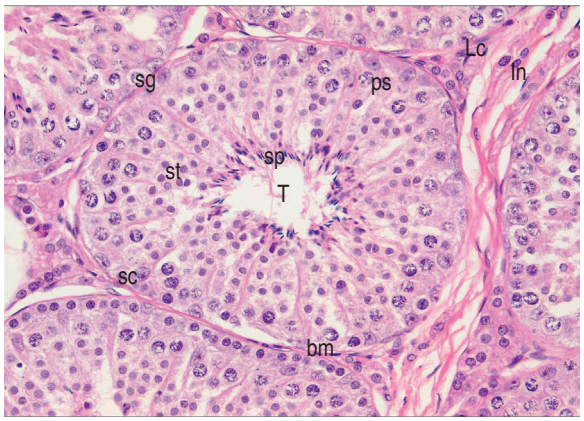
Blood and Nervous Supply to the Testis

The testes are supplied with blood through the spermatic arteries, which arise from the caudal aorta close to the renal arteries. In the domestic species these arteries pass through the inguinal canal, enclosed in peritoneum, forming a major component of the spermatic cord. In animals with scrotal testes the spermatic artery becomes highly convoluted from the point at which it passes through the inguinal canal, although the degree of convolution is less if the scrotum is inguinal than if pendulous. In the bull the spermatic artery in the spermatic cord is approximately 5 m in length (Setchell & Breed 2006). The artery does not divide until it reaches the testis (Setchell & Breed 2006), at which point it divides into major distributing arteries that run over the surface of the testis before descending into its parenchyma.

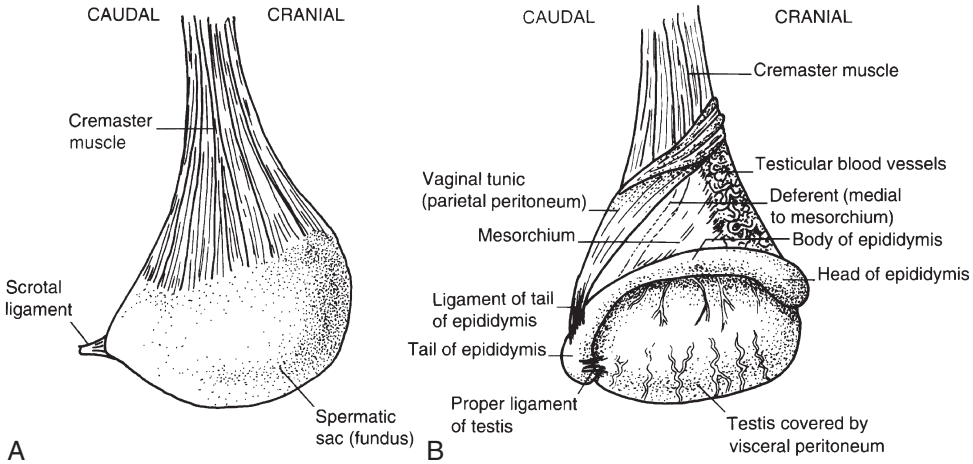
The testis is drained by an anastomosing plexus of veins (the pampiniform plexus) that arise in the tunica albuginea and return to the spermatic cord through the inguinal canal and thence to the caudal vena cava. Initially, many veins are identifiable in the plexus, but as the plexus ascends the spermatic cord, fewer and fewer collateral branches are apparent until a few main veins penetrate the inguinal canal. These finally join, as a single vein, with either the caudal vena cava or renal vein (Setchell & Breed 2006). In the bull, veins of different sizes are present in the plexus: large veins running parallel to each other and surrounding the spermatic artery, smaller veins with a less organised architecture, and tiny veins in or closely applied to the walls of the spermatic artery. All these veins communicate with one another (Hees et al. 1984), and there is also evidence of arteriovenous anastomoses within the spermatic cord.

The spermatic artery is therefore in very intimate contact with the pampiniform plexus. This complex vascular anatomy fulfils several functions. The extension of the length of the spermatic artery results in the arterial pulse being almost completely eliminated by the time the artery reaches the testis (Waites & Moule 1960). In the rat (Maddocks & Setchell 1988) the pulse is reduced from 34 mm Hg at the proximal end of the spermatic cord to 6 mm Hg at the testicular end. It appears that a pulsatile arterial blood supply to the testis is incompatible with normal spermatogenesis. Second, spermatogenesis is more efficient at temperatures lower than the mammalian core body temperature. The close apposition of artery and veins allows heat exchange to occur between spermatic artery and vein such that the temperature in the testis is several degrees lower than the core body temperature. Third, it is possible that some countercurrent exchange of small molecules such as testosterone may occur between spermatic vein and artery, although the importance of such transfers remains to be established.

The nervous supply of the testis is derived from the thoracolumbar sympathetic outflow, whose visceral motor fibres innervate the smooth muscle of the testicular arterioles and of the testicular and epididymal capsules. These fibres and their accompanying visceral sensory fibres run in the spermatic cord. The scrotum has both visceral and somatic innervation, which is derived from nerves that pass through the inguinal canal, and arise as branches of the pudendal nerve. A further prominent feature of the innervation of the scrotum is the motor supply to the cremaster muscle and dartos. However, as might be expected from the interspecies variation in anatomy of the scrotum, there is also considerable variation in the detail of its nervous supply.



• **Fig. 2.3** Histology of the testis. Interstitial tissue *(In)* containing Leydig cells *(Lc)*, blood, nervous tissue, and lymphatic tissue is interspersed between seminiferous tubules *(T)*, whose lumen is lined, in some sections, by formed spermatozoa *(sp)*. The periphery of the tubules is limited by a basement membrane *(bm)* and is composed of spermatogonia *(sg)* and Sertoli cells *(SC)*, with spermatocytes (primary spermatocytes *(ps)* are present in this section), and spermatids *(st)* occurring deeper in the tubules.



• **Fig. 2.4** Ligaments of the scrotum. (A) Lateral view of the right spermatic sac of the horse. (B) Lateral aspect of the contents of the right spermatic sac of the horse. The most ventral part of the spermatic sac has been incised so as to enter the lumen of the vaginal tunic. (Redrawn with permission from Cox 1982.)

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TABLE 2.1 Accessory sex glands of the main domestic species

Species	Ampulla	Prostate	Vesicular Gland	Bulbourethral Gland
Cat		++		++
Dog	(+)	+++		
Horse	++	++	++	+
Cattle	(+)	++	+++	+
Sheep	(+)	++	+++	+
Pig		+	++	+++

The relative size and importance is indicated by the number of + symbols. Ampullary glands are present in species marked (+) but are not anatomically prominent.

Accessory Glands

The accessory glands include the ampullae, prostate, vesicular glands, and bulbourethral (or Cowper's) glands. There is much variation between the anatomy of the accessory glands in the different species, which is summarised in Table 2.1.

Structure and Function of Spermatozoa

Spermatozoa are divided into three main segments: the head, midpiece, and tail (Fig. 2.5). The head consists of little other than the condensed nucleus and the overlying acrosome. Of the other enzymes contained within the acrosome, the main two are acrosin and hyaluronidase. During the acrosome reaction the outer acrosomal membrane fuses with the sperm membrane, under the control of intra- and extracellular calcium, whereupon exocytosis of the contents of the acrosome occurs (Harrison & Roldan 1990). The main functions ascribed to the acrosomal enzymes are dispersal of the cumulus oophorus and local lysis of the zona pellucida. The inner membrane of the acrosome is relatively stable and remains intact after the acrosome reaction has occurred; some of the acrosomal enzymes are probably bound to the inner acrosomal membrane (Ferrer et al. 2012). Penetration of the zona pellucida and fusion with the oolemma are both receptor-mediated events, with specific areas of the sperm head binding to target components of the oocyte (Wassarman 1990). The classical proposed sequence of acrosome reaction and fertilisation is that the acrosome reaction is initiated in the cumulus oophorus and that the action of the acrosomal enzymes plus the hypermotility of the capacitated sperm propels the sperm through the cumulus until it binds with the zona pellucida (Jin et al. 2011). However, there is evidence for zona-induced acrosome reaction in some species, whereas in others, induction of the acrosome reaction by the cumulus, rather than by the zona, may be required for successful fertilisation (Gadella 2013). Moreover, there is now evidence that acrosin, apart from any role it has in dispersal of the cumulus or zona, is also involved in the binding between the sperm head and the oolemma (Mao & Wang 2013).

The midpiece and tail of the sperm may be considered to form a single functional entity. The tail itself consists of a central axoneme, which, in the region of the midpiece, is sheathed in a helix of mitochondria (Bedford & Hoskins 1990, Amaral et al. 2013). Sperm metabolise simple molecules, principally sugars and their derivatives (e.g., fructose, glucose, mannose, and pyruvate), by both aerobic and anaerobic pathways to provide energy for motility and the maintenance of ionic gradients across membranes (Florman & Sulcibella 2006). Forward motility of sperm results from coordinated waves of flagellar bending progressing from the neck along the length of the tail. Bending of the tail occurs as the result of forces generated between adjacent peripheral doublets of the axoneme (Satir et al. 1981). The dynein arms of the doublet, which in the resting state are bound to the adjacent doublet, unbind, elongate, and then bind to a new site further along the filament. The unbinding process, which is the adenosine triphosphate (ATP)-using step, is then repeated, resulting in a progressive bending of the flagellum. The doublets on one side of the axoneme work in opposition to each other, providing the alternating beat of the tail. After capacitation the rate and amplitude of the flagellar beat greatly increases, and the rate of energy usage by the sperm is correspondingly elevated (Florman & Dulcibella 2006). The motility of the cell itself probably has little role in the movement of spermatozoa through the cervix and uterus, for this is accomplished mainly through contractions of the female genital tract (Hunter 1980). However, passage through the uterotubal junction and within the oviduct does require sperm motility, whereas the enhanced, whiplash motility of the capacitated sperm is necessary for penetration of the cumulus and zona pellucida.

Development of the Male Reproductive System

Very early in embryonic development, primordial germ cells start to develop in the yolk sac, under the control of growth factors from the bone morphogenic protein (BMP) series (Itman et al. 2006). Invagination of the hindgut during embryonic differentiation brings the cells passively into the body cavity of the embryo, but thereafter they start to actively migrate. Primordial germ cells enter the endoderm of the prospective hindgut, after which they enter into its surrounding mesentery. They then enter the dorsal mesentery, before moving into the developing gonads (Cantu & Laird 2017). Quite small numbers of primordial germ cells enter the genital ridge, where they cease migration, aggregate together, and enter a period of mitotic replication (Wilhelm et al. 2007). In rodents replication of these gonocytes is arrested during the latter stages of fetal development (Yao et al. 2003), resuming after birth, whereas in the fetal ram there appears to be continuous replication of gonocytes from Day 42 until birth (Hochereau-de-Reviers et al. 1995). Resumption of gonocyte replication in the rodent testis is accompanied by differentiation of gonocytes into spermatogonia (Itman et al. 2006). Meiosis does not commence until the onset of puberty (McLaren 1995). The somatic cells that will form the future Sertoli cells also migrate into the gonadal ridge from the coelomic epithelium, whereas the precursors of the interstitial and Leydig cells are derived from the underlying mesonephros (O'Shaughnessy et al. 2006). At this stage the gonad is bipotential, with its somatic components being capable of differentiating into the support and steroidogenic cells of either the male or female gonad (Merchant-Larios et al. 1993).

In male embryos, development of the presumptive gonad into a testis is stimulated by the activation of the sex-determining gene of the Y chromosome (*Sry*) in pre-Sertoli cells (Sinclair et al. 1990). Activation of *Sry* is dependent on the prior expression of several promotor genes in the undifferentiated gonad. *Sry,* once activated, through an interaction between steroidogenic factor 1

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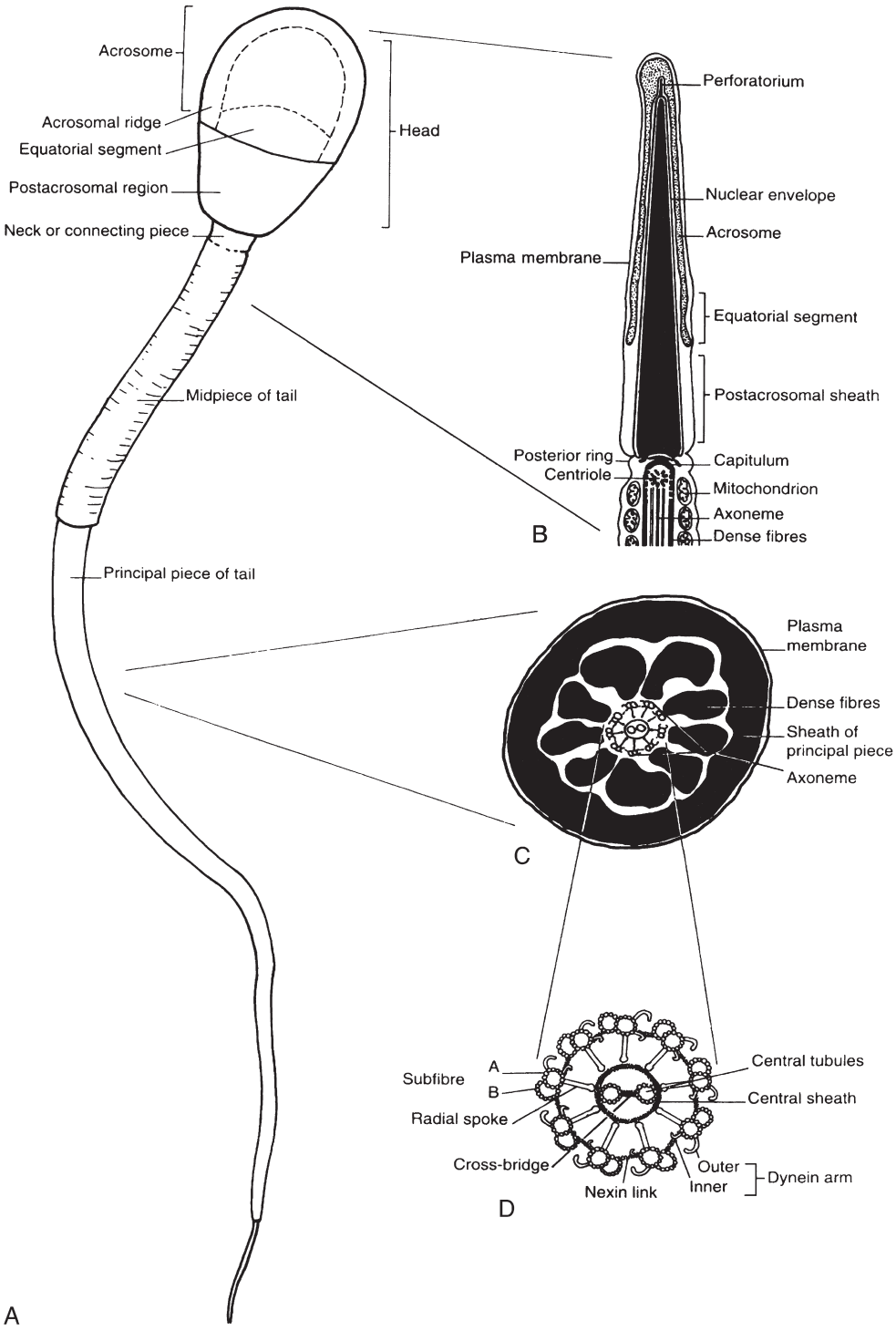

• **Fig. 2.5** Diagram of the ultrastructure of a spermatozoon. (A) Main structural regions as revealed by light microscopy. (B) Generalised ultrastructural features of the head and connecting piece. (C) Ultrastructure of the proximal principal piece of the tail. (D) Generalised detail of the ultrastructure of the axoneme of the tail. (Redrawn and adapted from Bedford & Hoskins 1990.)

(*SF1*), provides for the upregulation of the autosomal gene, *Sox9* (Gunes et al. 2016), within the prospective Sertoli cells. *Sry* transcription ceases soon after the onset of *Sox9* production in mice, although it continues for a little longer in ruminants (Parma & Radi 2012); as its expression declines, *Sox9* becomes the dominant hormone of sex determination. *Sox9* stimulates transcription of *Fgf9* (fibroblast growth factor 9), which in turn stimulates the proliferation of future Sertoli cells (Schmahl et al. 2004). Sertoli cells are therefore the first cell type to differentiate from bipotential supporting cell precursors within the gonad, and their presence is therefore the first indicator that the gonad has passed from the indifferent stage into testis development (Wilhelm et al. 2007). Thus the level of *Sox9* expression and the number of Sertoli cells appear to be critical for normal male differentiation (She & Wang

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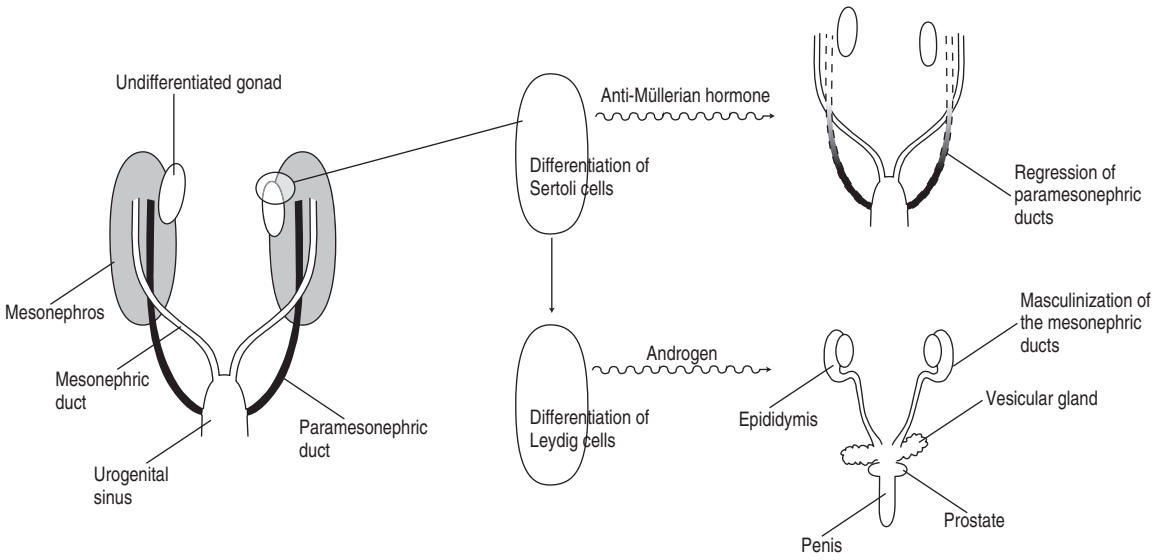

• **Fig. 2.6** Development of the male reproductive tract.

2017), at least partly due to the need to maintain expression of *Sox9* through a positive feedback mechanism. *Sox9* also stimulates the expression of prostaglandin D synthetase; prostaglandin D promotes the uptake of *Sox9* into the nucleus, where it binds to its own promotor to maintain its expression (Windley & Wilhelm 2015).

The onset of *Sry* expression is associated with migration of further cells from the mesonephros that will become the myoid cells of the seminiferous tubules (Capel et al. 1999). Differentiation of Sertoli cells and migration of myoid cells results in the development of testis cords, which initially appear as groups of germ cells surrounded by Sertoli cells, which are further surrounded by peritubular myoid cells. It appears that both Sertoli cells and myoid cells are needed for the normal development of testis cords (Buehr et al. 1993). These cords will eventually become the seminiferous tubules of the mature testis (Tilmann & Capel 1999).

Products of the Sertoli cells and Leydig cells are thereafter responsible for the differentiation of the remainder of the male reproductive tract. *Sox9,* derived from the Sertoli cell, initiates the expression of a series of genes that regulate the differentiation of the male reproductive system. *Sox9* and *SF1* act synergistically to initiate expression of *Amh* (anti-Müllerian hormone, also known as Müllerian inhibiting substance), which is responsible for the regression of the paramesonephric ducts (Swain 2006). *Amh* is a large (~140 kDa), dimeric protein (Swain 2006) that belongs to the TGFβ superfamily (Wilhelm et al. 2007). Its actions are primarily paracrine, acting through receptors in the mesenchymal cells that surround the paramesonephric duct, resulting in apoptosis of the epithelial cells of the paramesonephric ducts (Roberts et al. 2002). *Fgf9* and *Sox9* also act to suppress the expression of female-specific genes (Eggers & Sinclair 2012). *Amh* also has a positive role in male development inasmuch as it is required for normal development of the efferent ducts (Behringer et al. 1994).

Desert hedgehog (*Dhh*), which is secreted by the Sertoli cells, has many morphogeneic effects, acting through its receptor PTCH1 to induce differentiation of peritubular myoid cells and Leydig cells (Clark et al. 2000). Myoid and endothelial cells also play a role in directing the differentiation of Leydig cells (Kitamura et al. 2002). Different populations of Leydig cells are present in the fetus and adult, which are dissimilar to each other in terms of the steroids that they produce and the regulation of their steroidogenesis. Precursors of fetal Leydig cells appear to comigrate from the mesonephros with the cells that will become the steroidogenic cells of the adrenal (O'Shaughnessy et al. 2006). Multiplication and differentiation of these precursor cells are under the control of the Sertoli cells, through signalling molecules such as *Dhh* (Yao et al. 2002) and platelet-derived growth factor (Brennan et al. 2003). Fetal Leydig cells differ from those of the adult, inasmuch as their primary steroid secretion is androstenedione rather than testosterone, which has to be converted into testosterone in the seminiferous tubules (O'Shaughnessy et al. 2000). In the mouse, fetal Leydig cell steroid secretion occurs without a requirement for trophic hormones, although it can be stimulated by both luteinising hormone (LH) and adrenocorticotrophic hormone (ACTH) (O'Shaughnessy et al. 2005).

Furthermore, Leydig cells produce androgens, which stimulate the development of the mesonephric (Wolffian) ducts (Fig. 2.6) to form the tubular parts (including epididymis, ductus deferens, ampulla, vesicular gland, and prostate) of the male genital tract. Androgens are also responsible for the differentiation of the penis from the genital tubercle (Swain 2004). Exposure of the female fetus to androgens results in masculinisation of the internal genitalia, with development of the mesonephric ducts and atrophy of the paramesonephric ducts, and a greater or lesser degree of masculinisation of the genital tubercle (Jost 1953). It also causes masculinisation of the hypothalamopituitary endocrine axis (Robinson et al. 2003). Evidence of this masculinisation can be seen in genotypic female animals that have been exposed to androgens *in utero* (e.g., the freemartin ewe: Parkinson et al. 2001 and see Chapter 9).

Descent of the Testis

As the testis develops in the mesonephros, differentiation of the definitive kidney (the metanephros) begins, and as it grows, the metanephros replaces the mesonephros as the organ of osmoregulation. The testis then migrates from its position within the abdomen towards its definitive position in the scrotum in a process referred to as the descent of the testis. The testis traverses the abdomen (Gier & Marion 1970; Fig. 2.7 A, B) partly (i) as a result of differential growth between the pelvis, abdomen, kidney, and testis; (ii) by

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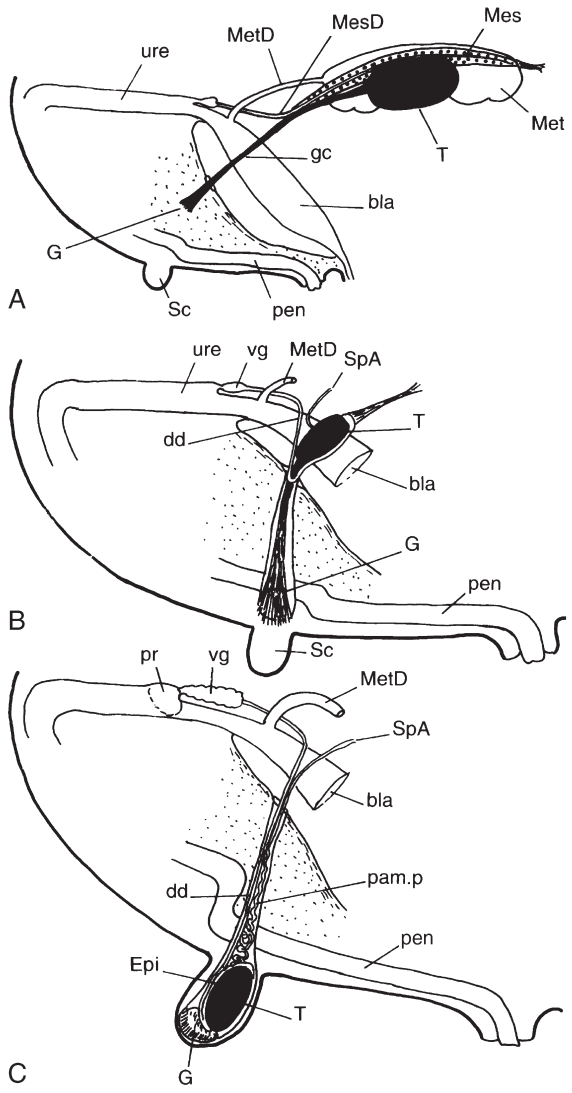

• **Fig. 2.7** Descent of the testis in the bull. (A) 65-day fetus, (B) 96-day fetus, and (C) 140-day fetus. The changing relationships between the gubernaculum *(G),* testis *(T),* and vaginal tunics will be noted. *bla,* Bladder; *dd,* ductus deferens; *Epi,* epididymis; *gc,* gubernacular cord; *Mes,* mesonephros; *Met,* metanephros; *MetD,* metanephric duct; *pam.p,* pampiniform plexus; *pen,* shaft of penis; *pr,* prostate; *Sc,* scrotum; *SpA,* spermatic artery; *ure,* urethra; *vg,* vesicular gland. (Redrawn with permission from Gier & Marion 1970.)

the action of the gubernaculum; and (iii) as a result of regression of the cranial suspensory ligament of the gonad, which holds the urogenital tract near the developing diaphragm (Mamoulakis et al. 2015).The transabdominal phase of testicular descent does not appear to be under androgen control, except that regression of the cranial suspensory ligament is androgen-dependent, whereas the inguinal–scrotal phase (Fig. 2.7C) requires androgen. Insulin 3 (*syn:* insulin-like hormone 3), derived from Leydig cells, appears to be the key regulator of testicular descent; the transabdominal phase of descent and the development of the gubernaculum appear to be critically dependent on it (Verma-Kurvari et al. 2005), and there is good evidence that it is also involved in the inguinal–scrotal (Kaftanovskaya et al. 2011) phase of descent. Genetic deficiencies of insulin 3 production at the time of the descent of the testis appear to be a major component of the inherited basis of cryptorchidism,

TABLE 2.2 Age at which testes descend into the scrotum

Species	Time of Testicular Descent
Cat	2–5 days after birth
Dog	Between the last few days of gestation and the first few days after birth
Horse	Between 9 months of gestation and a few days after birth
Cattle	3.5–4 months of gestation
Sheep	Midgestation (≈80 days)
Pig	After 85 days of gestation

whereas oestrogen-induced impairment of insulin 3 may be of significance as an environmental cause.

Before the testis itself passes through the inguinal canal, the canal itself is distended by a thickening of the gubernaculums and by the passage of the tail of the epididymis. Thus the gubernaculum, tail of epididymis and tip of the vaginal process precede the testis through the inguinal canal. Final passage of the testis, in the inguinal–scrotal phase of the descent, requires androgen; however, the target of this androgen is not the gubernaculum but, rather, appears to be the nucleus of the genitofemoral nerve within the CNS (Hutson & Beasley 1987). This nucleus releases CGRP (calcitonin gene-related peptide) that, in rodents, acts on the muscular component of the developing gubernaculum/cremaster muscle to produce contraction upon the descending testis (Momose et al. 1992). In the latter stages of the descent of the testis, the gubernaculum shrinks concurrently with the testicular descent. It remains controversial as to whether this shrinkage 'pulls' the testis into the scrotum, although the changes of structure of the gubernaculum over the period of testicular descent (i.e., increase in connective tissue and a marked increase in collaged and elastic tissue) strongly suggest that its role in testicular descent is an active one (Favorito et al. 2014). Thus the mechanisms of the descent of the testis are complex, involving: differential growth of the pelvis, abdomen, and kidney; pressure from the abdominal viscera upon the testis; and a role of the gubernaculum. The latter, although clearly critical, still has to be fully elucidated. The times at which the testis is first present within the scrotum are given for the main domestic species in Table 2.2.

Physiology of the Testis

Endocrinology

Male reproductive physiology is under the primary endocrine control of the pituitary gonadotrophins, luteinising hormone (LH), and follicle-stimulating hormone (FSH), both of which are synthesised and secreted in response to gonadotrophin-releasing hormone (GnRH). At a local level, many of the functions of the testes are also regulated by intratesticular paracrine and autocrine factors, as well as being influenced by a number of endocrine metabolic regulators.

The secretion of LH is pulsatile in response to episodic stimulation of the pituitary by GnRH, resulting in irregular episodes of LH secretion occurring every 2 to 4 hours. The primary action of LH is upon the Leydig cells, in which it controls testosterone synthesis.

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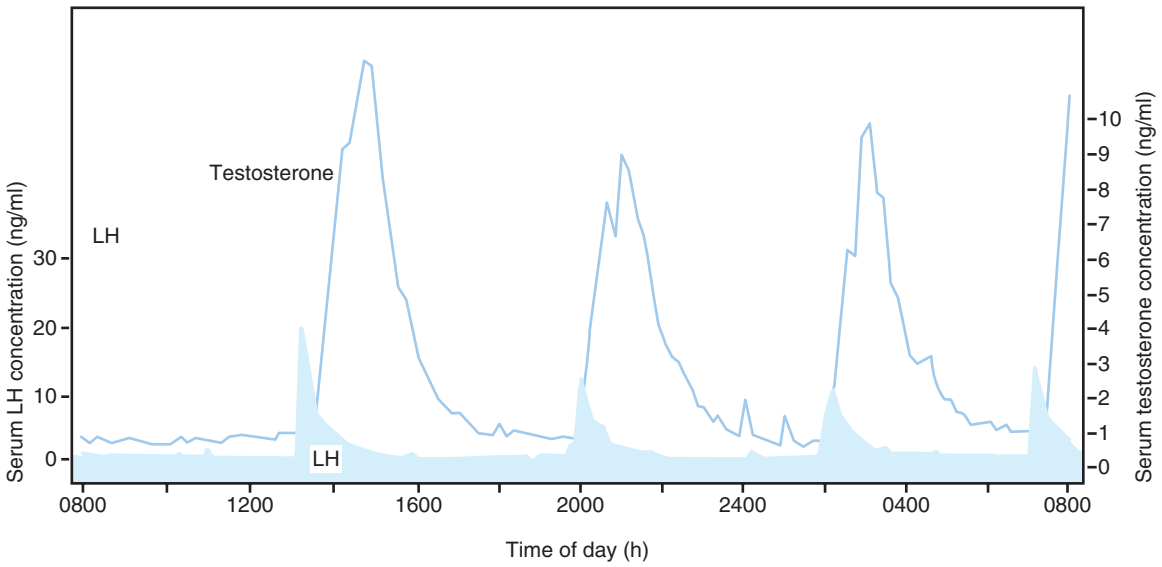

• **Fig. 2.8** Typical profile of the patterns of LH and testosterone secretion in the ram. (Redrawn from D'Occhio et al. 1982a.)

In fact, it appears that regulation of testosterone synthesis is the only indispensable function of LH within the testis (Holdcraft & Braun 2004). Peak testosterone concentrations follow those of LH by about 40 minutes and decline to prestimulation values over a further 40 to 80 minutes (D'Occhio et al. 1982a; Fig. 2.8).

LH receptors are primarily present on Leydig cells, although there is also evidence of their presence in germ cells (Lei et al. 2001). Experiments upon hypophysectomised rams suggest that the role of these receptors may be to control the rate of division of stem spermatogonia (Courot & Ortavant 1981). The actions of LH upon the Leydig cell are mediated through adenylate cyclase, guanylate cyclase, and calcium channels (Tremblay 2015) that, through a series of intermediate steps, regulate steroidogenesis by controlling the conversion of cholesterol into pregnenolone (Stocco & McPhaul 2006). This conversion is the rate-determining step of testosterone synthesis. It, in turn, is regulated by the activity of steroidogenic acute regulatory protein (*StAR*), which controls the rate of cholesterol uptake into mitochondria (i.e., into the site of steroid synthesis). Activation and expression of *StAR* requires both chronic and acute stimulation by LH (Shiraishi & Matsuyama 2017): hence, the necessity for continuous pulsatile secretion of LH. A number of other growth factors and cytokines have been demonstrated to have positive**,** and negative**,** modulatory effects upon the action of LH upon Leydig cells (Bornstein et al. 2004). Among these the effects of growth hormone (Colon et al. 2005), insulin-like growth factor-1 (IGF-I) (Abo-Elmaksoud & Sinowatz 2005), insulin (Ballester et al. 2004), and fibroblast growth factor 9 (*FGF9:* Lin et al. 2010) appear to be of importance in stimulating testosterone synthesis; others (e.g., *Amh* and tumour necrosis factor–α) have inhibitory effects.

Negative-feedback regulation of LH secretion is mediated by the effects of testosterone upon hypothalamic GnRH and by pituitary synthesis and secretion, both directly and after conversion to dihydrotestosterone or aromatisation to oestradiol (O'Donnell et al. 2006). Curiously, in long-term castrated ruminants neither negative feedback nor libido can be restored by testosterone administration, for brain aromatase activity is eventually lost and oestrogen itself has to be given for the restoration of these effects (D'Occhio et al. 1982b).

Testosterone is required for the production of spermatozoa and their subsequent maturation in the epididymis, as well as for the function of the accessory sex glands and for the development of masculine secondary sexual characteristics. Within the testis, androgen receptors are present in Leydig, Sertoli, and myoid cells (Holdcraft & Braun 2004) but not in germ cells. Testosterone is converted by 5α–reduction into 5α–dihydrotestosterone (DHT) in Sertoli cells and accessory sex glands. DHT, which is not susceptible to aromatisation and is a more potent androgen than testosterone itself, appears to be the primary androgen controlling accessory sex gland activity, whereas testosterone is the primary androgen involved in spermatogenesis (Walker & Cheng 2005). Both testosterone and DHT are bound within the tubule lumen by androgen-binding protein (ABP), which is secreted by the Sertoli cells. The role of ABP therefore appears to be to maintain high androgen concentrations in the lumina of the seminiferous tubule and epididymis.

The main target of FSH is the Sertoli cell, in which it also acts through adenylate–cyclase-linked enzyme systems. Under the influence of FSH, Sertoli cells secrete ABP (Gunsalus et al. 1981) and aromatise testosterone into oestrogens (Setchell et al. 1983); FSH is also responsible for switching on a great many genes in the Sertoli cell that regulate or support spermatogenesis (Walker & Cheng 2005). Most aspects of spermatogenesis require support by FSH and/or testosterone. However, it appears from genetic and pharmacological studies in rodents that the primary role of FSH in spermatogenesis is stimulation of Sertoli cell proliferation during prepubertal development (Heckert & Griswold 2002), and, because the number of Sertoli cells largely determines the number of germ cells (O'Donnell et al. 2006), this action is an important determinant of postpubertal spermatogenic yield (Holdcraft & Braun 2004).

It is generally considered that the secretion of FSH lacks the episodic pattern that is characteristic of LH, exhibiting longer term fluctuations instead. However, there are some reports of a more clearly pulsatile pattern of FSH secretion. Negative feedback is by both gonadal steroids and inhibin. Inhibin is produced by Sertoli cells (Baird et al. 1991) and causes negative feedback on FSH secretion, directly at the level of the pituitary (Tilbrook et al.

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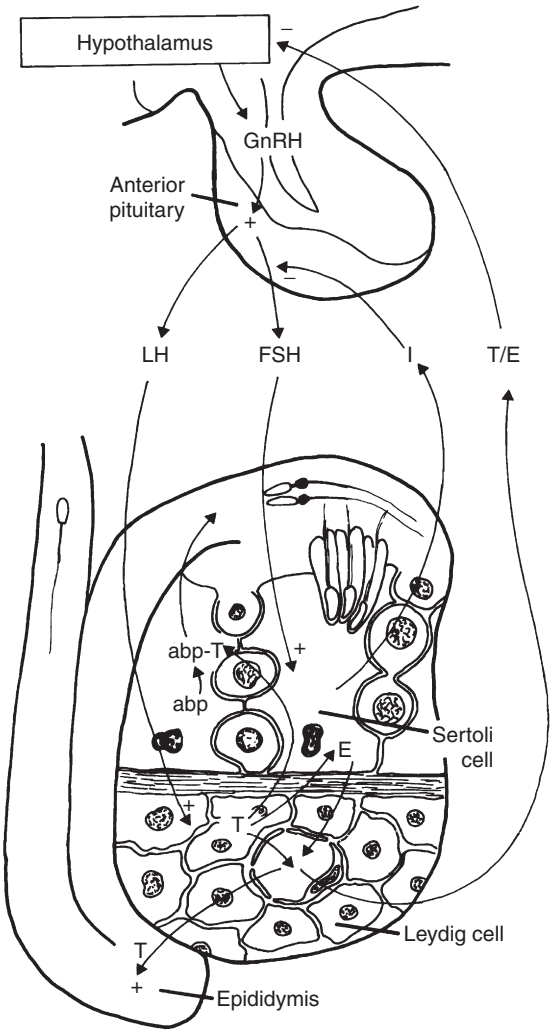
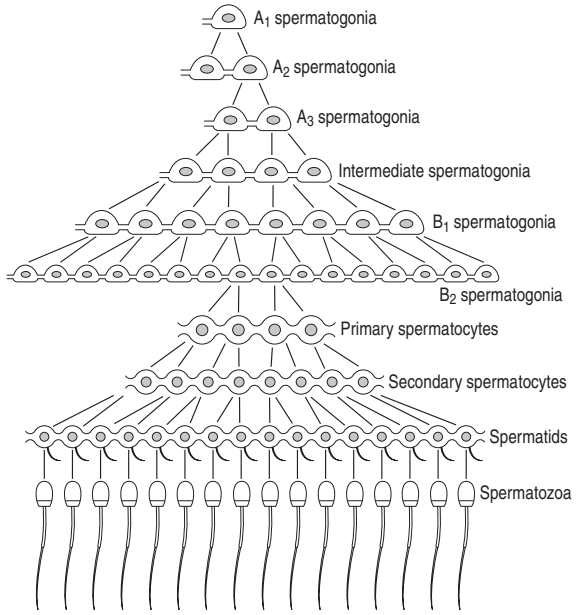


• **Fig. 2.9** Diagram of the endocrine relationships controlling the testis. *abp,* Androgen-binding protein; *E,* oestrogen; *GnRH,* gonadotrophin-releasing hormone; *I,* inhibin; *T,* testosterone. (Redrawn and adapted from Amann & Schanbacher 1983.)



• **Fig. 2.10** Multiplication of cells during spermatogenesis in the bull. A_1 spermatogonia undergo a series of mitotic divisions to produce A_2, A_3, intermediate, B_1, and B_2 spermatogonia. The final mitotic division produces primary spermatogonia, which enter meiosis, producing secondary spermatocytes after the first meiotic division and spermatids after the second. Spermatids then differentiate into spermatozoa without further cellular division.

1993); it has little effect on LH (Tilbrook et al. 1995). Inhibin B is the predominant form (Phillips 2005) in the males of most domestic species, although in the ram it is inhibin A (McNeilly et al. 2002). Activin, which is also produced by the Sertoli cell, stimulates FSH production in the pituitary, although most of its functions actually seem to be as a paracrine agent within the testis (de Kretser et al. 2004). A schematic summary of the main endocrine relationships of the testis is given in Fig. 2.9 (Amann & Schanbacher 1983).

Spermatogenesis

Spermatogenesis is the basic process of male reproduction, resulting in the production of spermatozoa. It occurs in the seminiferous tubule of the adult testis and comprises three main processes: mitotic replication of spermatogonia, meiosis, and postmeiotic differentiation into spermatozoa (Fig. 2.10).

These processes of spermatogenesis are reflected in the functional morphology of the seminiferous tubule (Courot et al. 1970). The seminiferous epithelium appears as concentric layers of spermatogonia, spermatocytes and spermatids, with characteristic associations between generations of cells throughout the depth of the seminiferous epithelium (Fig. 2.11). Cellular associations are generally classified into Type I, in which two generations of primary spermatocytes and one of spermatids are present, and Type II, in which there is only one generation of primary spermatocytes but two of spermatids. Transition between Type I and Type II occurs after the maturation divisions, whereas Type II changes into Type I with the release of spermatozoa and the arrival of a new generation of spermatocytes from the last spermatogonial division. Each generation of seminiferous cells is linked by cytoplasmic bridges, so that developmental stages are synchronous within each generation, and substantial areas of seminiferous epithelium exhibit cells at a similar stage of development. Fibroblasts and myoid cells surround the basement membrane of the seminiferous tubule externally. The blood supply is limited by the basement membrane and does not pass into the tubule itself.

Within the tubule, there are somatic Sertoli cells and the various stages of the seminiferous cell line, which together form the seminiferous epithelium. Sertoli cells, which rest upon the basement membrane, are irregularly cylindrical in shape and have large, variably shaped nuclei situated close to the basement membrane. They multiply during fetal and prepubertal life (Wilhelm et al. 2007) in a process that is dependent on FSH and activin (Boitani et al. 1995) but is independent of testosterone. It is generally accepted that the full complement of Sertoli cells is present at the time of puberty, although there is some evidence for an annual cycle of loss and replication of Sertoli cells in at least some seasonally breeding species (Johnson & Thompson 1983, Hochereau-de-Reviers et al. 1987). Sertoli cells extend through the entire thickness of the seminiferous epithelium, so that the germinal cells in all stages of spermatogenesis are in close contact with them. As Sertoli cells are the only somatic cells that are in direct contact with differentiating germ cells, they have to provide both physical

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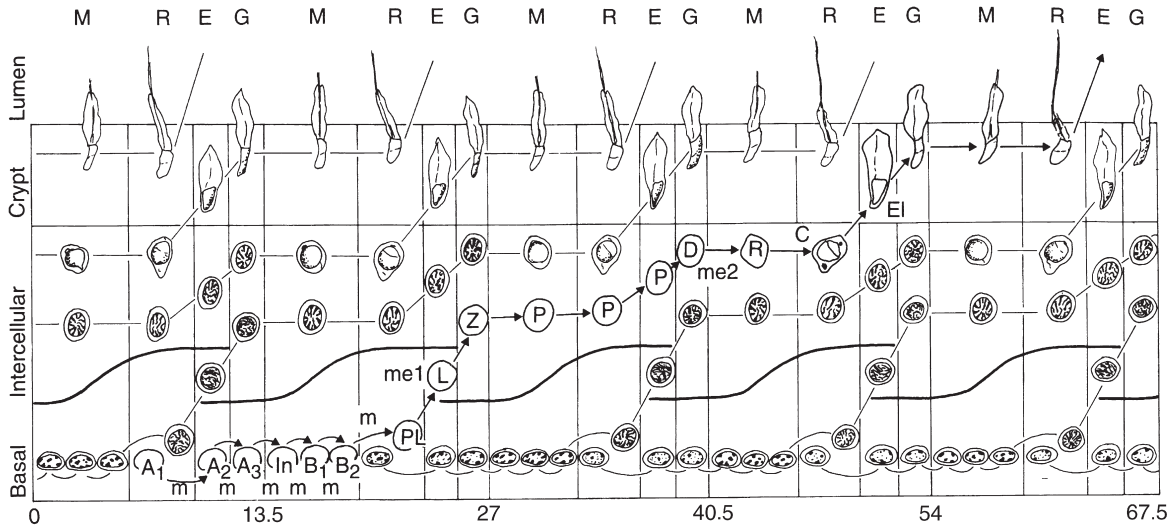

• **Fig. 2.11** Spermatogenesis in the bovine testis. Stem cells undergo mitotic *(m)* divisions producing successive generations of diploid spermatogonia (A_1, A_2, A_3, intermediate (In), B_1, and B_2), before entering the first meiotic division *(me1).* Preleptotene primary spermatocytes *(PL)* pass through the successive stages of the long first meiotic prophase (*L,* leptotene; *Z,* zygotene; *P,* pachytene; *D,* diakinesis), becoming short-lived secondary spermatocytes. These proceed through the second meiotic division *(me2),* producing early round spermatids *(R).* These differentiate into spermatozoa without further division, after nuclear condensation *(C)* and elongation *(El).* The solid horizontal line indicates the position of tight junctions between Sertoli cells: the site of the blood–testis barrier. Four major groupings of cells can be determined, based upon the morphology of the spermatids/spermatozoa: *E,* elongation; *G,* grouping; *M,* maturation; *R,* release. These recur every 13.5 days. (Adapted from an original drawing by Brian Setchell.)

and nutritional support for spermatogenesis (Griswold 1998). The seminiferous tubules are divided into basal and luminal compartments by junctional complexes (tight cell junctions: TCJ) between adjacent Sertoli cells (Setchell & Breed 2006). These junctions form the blood–testis barrier (Stocco & McPhaul 2006), which maintains the specialised environment of the lumen of the seminiferous tubules and separates meiotic and postmeiotic (haploid) cells from the immune system. The blood–testis barrier is not only a physical barrier, but also a functional barrier: Stanton (2016) described its functions as being a physical barrier, a physiological barrier of cell transporters that control movement of substances to and from the lumen, and an immunological barrier comprising immuno–regulatory factors and tolerance mechanisms. The formation of the blood–testis barrier is androgen-dependent, as is the adhesion between germ cells and Sertoli cells (Holdcraft & Braun 2004), and first forms at around the time of puberty.

FSH and testosterone are essential for the normal activity of Sertoli cells. Although many aspects of the Sertoli cell support of spermatogenesis can be maintained by either hormone (Walker & Cheng 2005) (probably though activation of similar intracellular pathways), normal spermatogenesis cannot be completed in the absence of androgen. Some aspects of Sertoli cell function appear to have an absolute requirement for FSH because, in the sheep, some spermatogonial divisions can only be supported by FSH (Kilgour et al. 1998). However, the main hormone responsible for maintenance of spermatogenesis in the postpubertal animal is testosterone (O'Donnell et al. 2006). Many other substances contribute to the regulation of Sertoli cell activity, particularly IGFI, IGFII, insulin, activins, follistatin and inhibin, and BMPs (Itman et al. 2006, Dimitriadis et al. 2015). In response to stimulation by these agents, Sertoli cells secrete oestrogens, inhibin, a GnRH-like peptide, proteins (including ABP), lactate, pyruvate, and tubule fluid. Significant changes to the morphology and activity of Sertoli cells occur in the peripubertal period, associated with the onset of spermatogenesis, although there are also annual changes in their morphology in seasonally breeding species according to the activity of the gonadotrophic axis (Hochereau-de-Reviers et al. 1985).

After the migration of the primordial germ cells into the genital ridge, they replicate and differentiate into spermatogonia. In the mature animal, spermatogonia are divided into A, intermediate, and B classes, with each class further subdivided according to morphology and degree of differentiation (Fig. 2.11). Thus in the bull and ram, A_0, A_1, A_2, A_3, intermediate, B_1, and B_2 spermatogonia are present (Hochereau-de-Reviers 1976). A-series spermatogonia are the least differentiated and form the reservoir of stem cells within the seminiferous tubule. It is likely that stem cells are regenerated by asymmetrical divisions of early A-series spermatogonia, with one progeny cell remaining as an uncommitted stem cell and the other being committed to undergo further mitotic and meiotic divisions. In mice (see Chen & Liu 2015), these A-series spermatogonial stem cells are divided into A_{single} and A_{paired} or $A_{aligned}$, such that the former are true stem cells and the latter two are committed to enter the cascade of mitotic divisions. Various transcription factors are present in A_{single} that are not present in more committed/differentiated spermatogonia, and which disappear as spermatogonia progress from the progenitor pool into the differentiating pool. Griswold (2015) considered that retinoic acid is the key extrinsic factor that controls the entry of A spermatogonia stem cells into the developing pool of the mouse testis. The onset of meiosis in prespermatocyte germ cells is similarly signalled by Sertoli cell-controlled paracrine mechanisms. Two of these mechanisms are worthy of mention: the copromotion of meiosis by retinoic acid (the active form of Vitamin A) and FSH (Zhang et al. 2011); and the promotion of meiosis by androgen–androgen receptor interactions, both within the Sertoli cell. Mutations in the androgen receptor

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are commonly associated with XY-sex reversal or nonspecific male infertility (Gottlieb et al. 2012).

All spermatogonia remain in contact with the basement membrane, but the area of contact is significantly reduced in B spermatogonia (de Kretser & Kerr 1994). As the final meiotic division of spermatogonia give rise to the primary spermatocytes, the cytoplasm of the Sertoli cells intervenes between the basement membrane and the primary spermatocytes. DNA synthesis occurs during mitotic divisions and then, to its greatest extent, during the formation of tetraploid nuclei during meiosis (Stocco & McPhaul 2006). RNA synthesis occurs during preleptotene and late pachytene (Kierszenbaum & Tres 1974). The first meiotic division then proceeds through the highly sensitive zygotene and pachytene stages. The pachytene stage is particularly sensitive to noxious damage such as by high testicular temperature or inadequate hormonal support. During the first meiotic division, the cells move deeper into the seminiferous epithelium, and the tight cell junctions of the Sertoli cells form beneath the spermatocytes and degenerate above them (Russell 1977, 1978), so that the cells effectively pass through the blood–testis barrier. The mechanism by which leptotene spermatocytes 'move through' the blood–testis barrier appears to involve a number of phases. These include: transient formation of an intermediate compartment enclosing the early leptotene spermatocytes; the progressive opening of tight junctions above migrating germ cells that then close below them once migration is completed; and the proliferation of tight junction components for assembly by an endocytic pathway (Stanton 2016). The TCJs themselves appear to be regulated by the stimulatory effects of androgen and retinoic acid and by the inhibitory effects of activin and TGFβ3, which, in turn, relates to the assembly of the 'new' TCJ below the spermatocyte and the disassembly of the 'old' TCJ above it (Nicholls et al. 2013). The role of FSH in barrier development is less well understood, with evidence for and against its direct involvement.

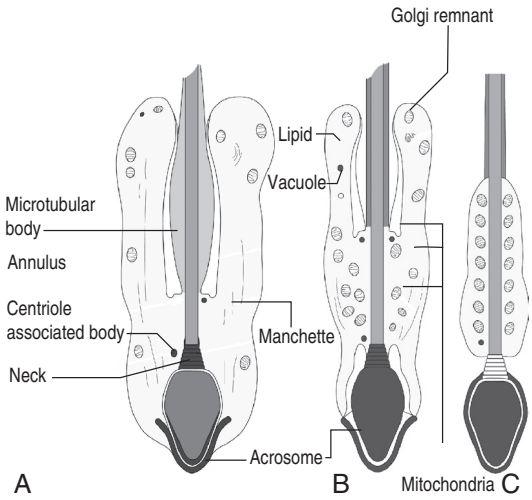
The consequence of this activity is that the progeny of the first meiotic division – the secondary spermatocytes – have moved from the basal to the luminal compartment of the seminiferous epithelium, and are thereafter separated from the general tissue fluid compartment. The second meiotic division produces spermatids, which do not divide further. At the end of meiosis, spermatids are round cells with round nuclei. Thereafter, they undergo marked changes in cell function and morphology that are associated with differentiation into spermatozoa (spermiogenesis).

Spermiogenesis

Immediately after completion of meiosis, the spermatids undergo a period of RNA synthesis, which is then followed by the beginnings of nuclear chromatin condensation (Monesi 1971). During the compaction of the nuclear chromatin, there is an abrupt change from a histone-based structure to a protamine-based structure (Rathke et al. 2014); the protamine-based structure is essential for normal fertility in mice. Acrosomal contents are concurrently synthesised in the Golgi complex. Vesicles derived from the Golgi fuse over the nuclear membrane overlying the basal pole of the nucleus (Courtens 1979), with further material subsequently being transported into the developing acrosome (Eddy 1994). The nucleus moves from a central position towards the margin of the cell, at the point at which the acrosome is developing. As the nucleus condenses and elongates, it shrinks significantly in size, resulting in redundancy of nuclear membrane.

The transient microtubular structure, the manchette, which appears in the postnuclear cytoplasm of the elongating spermatid during nuclear condensation, plays a key role in the shaping of the nucleus and in the formation of the tail. The manchette consists of microtubules and actin filaments which are connected and stabilised by the perinuclear ring (Lehti & Sironen 2016). Abnormal manchette formation is associated with abnormal development and shape of the sperm head (Calvi et al. 2015); the manchette has a key role in the development of the tail, particularly by the transport of proteins required for tail elongation (Kierszenbaum 2001). The tail is formed from the centrioles, one of which forms the connecting piece of the neck of the sperm, the other the axial filament of the tail (Eddy 2006). The structure of the axial filament (see later in this chapter) is common to flagella and cilia. The outer dense fibres are not derived from the centriole, although they are attached to the outer microtubules of the axial filament (Fawcett & Phillips 1969). The development of the midpiece occurs late in the process of spermatogenesis, as a helix of mitochondria condenses around the proximal part of the flagellum (Fig. 2.12). This process cannot take place until the manchette has disappeared. During formation of the acrosome and flagellum, the cytoplasm of the spermatid is deeply invaded by a process of the Sertoli cell, which extends between the forming flagellum and the residual cytoplasm. Finally, most remaining cytoplasm is engulfed by the Sertoli cell (Fouquet 1974) as the formed spermatozoon, with its remnant cytoplasmic droplet, is expelled from the crypt of the Sertoli cell into the lumen of the seminiferous tubule (spermiation). The mechanism of spermiation is not fully resolved; however, there is evidence that remodelling of the actin in and around the cell junctions between the Sertoli cell and the spermatid is at least one element in the process (Cheng & Mruk 2011).

The duration of spermatogenesis, i.e., the time between spermatogonial divisions and the release of the spermatozoon, is approximately 60 days in most domestic animals. Epididymal transit takes a further 8 to 14 days. Thus the interval between the most sensitive stage of spermatogenesis, meiotic prophase, and ejaculation is approximately 30 days (Amann & Schanbacher 1983). Hence the interval between damage to the testis and the appearance of abnormal spermatozoa in the ejaculate is generally between 30 and 60 days, depending on the site of damage.



• **Fig. 2.12** Formation of spermatozoa within the luminal crypts of the Sertoli cells. After formation of the acrosome and condensation of the nucleus at the basal pole of the developing sperm (A), there is a progressive alignment of the mitochondria around the flagellum (B) until, in the fully formed sperm (C), the helical sheath of mitochondria that makes up the midpiece is fully developed.

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Physiology of the Epididymis

When sperm are released from the seminiferous epithelium, they are immature, lacking normal motility and being incapable of fertilising. They acquire these abilities in their transit through the epididymis (Amann 1987, Eddy 2006); because the genome of the spermatozoon is almost completely inactivated, these changes are dependent on posttranslational modifications of the cell (Baker 2016). The epididymis is highly androgen-dependent; thus if androgen production is suppressed, epididymal function is immediately impaired. Likewise, the embryonic and postnatal development of the epididymis is highly dependent on androgen, mediated through androgen receptors and paracrine growth factors to induce differentiation and proliferation of the epididymal epithelium (Murishima et al. 2014). Dihydrotestosterone appears to be of greater importance than testosterone in the adult epididymis; high levels of 5α–reductase are present in the epididymis, especially in its initial segment. Interestingly, however, the activity of the efferent ducts, in which resorption of seminiferous tubule fluid takes place, is coregulated by oestradiol (Lee et al. 2000).

Anatomically, the epididymis can be considered to consist of four regions: the initial segment, head (caput), body (corpus), and tail (cauda). Functionally, it is divided into two major regions: a proximal region, which is involved in sperm maturation, and a distal region, which is concerned with storage and transport (Jones 1998a). The proximal segment has a relatively narrow lumen, an actively secretory epithelium and relatively sparse sperm population. The distal segment has a larger lumen, a much more prominent muscle layer surrounding the tubule, and a dense population of sperm. Studies in rodents suggest that each segment is further divided into several physiological compartments (Domeniconi et al. 2016), which differ widely from each other in terms of protein secretion and in their roles in the eventual development of functionality of the sperm. At the cellular level, the pseudostratified epithelium that lines the epididymal tubule includes**:** principal, narrow, clear, and basal cells. Principal and basal cells are present throughout the epididymis; narrow cells are located exclusively in the initial segment; and clear cells are present in the caput, corpus, and cauda of the epididymis (Breton et al. 2016).

Spermatozoa take between 8 and 14 days to traverse the epididymis, according to species. In the bull, sperm take 5 days to pass through the initial segment, head (caput), and body (corpus) of the epididymis and a further 5 to 9 days to traverse the epididymal tail (cauda). The transit time of the proximal segment is fixed, but it is variable in the distal segment of the epididymis because of its dual functions of maturation and storage. Hence, in periods of high ejaculation frequency, the passage time of the tail may be reduced**,** and relatively immature sperm may be ejaculated (Robaire et al. 2006). Movement of sperm through the epididymis is a passive process, which relies on the activity of the epididymal tubule rather than on the motility of the sperm (Robaire et al. 2006).

The protoplasmic remnant, which is initially sited close behind the sperm head, migrates distally to the end of the midpiece, before being finally shed in the tail of the epididymis. Sperm are immotile in the head of the epididymis but acquire the capacity for motility as they pass through its body. Actually, the environment within the lumen of the epididymal duct is maintained at a low pH and low bicarbonate concentrations by the actions of the clear cells as a means of maintaining sperm in a dormant state during their passage of the epididymis (Breton et al. 2016); thus the acquisition of motility is due in part to the epididymal environment. Indeed, a rise in pH and bicarbonate concentrations may occur in the preejaculatory phase, as a 'priming' of the sperm for activity after ejaculation. In the head of epididymis, sperm do not have the ability to fertilise, but this is acquired during passage through the epididymal body.

Less obvious, but of equal or greater importance to the morphological changes exhibited by sperm during their passage through the epididymis, are the changes in their plasma membrane, to which surface glycoproteins are added or modified by epididymal secretions and luminal cells. It is likely that these act to stabilise the acrosome when the sperm is within the female genital tract to reduce the surface immunogenicity of the sperm and to enhance the ability of the sperm membrane to bind to the zona pellucida (Amann 1987, Hammerstedt & Parkes 1987). During transit through the epididymis, sperm undergo changes in morphology, metabolic processes, activity of the flagellum, and the ability to bind to the zona pellucida (Eddy 2006). These changes are largely brought about by changes to the surface membrane of the sperm in response to substances, especially proteins, which are produced by the epididymis. The most active segment of the epididymis for protein secretion is the initial segment (Dacheux et al. 2003), whereas little protein is secreted in its distal segment. Some proteins are transferred to sperm by secretion into the lumen; others appear to be transferred via the apocrine secretion of membrane vesicles (Cooper 1998), whereas other proteins are lost from, or modified within, the sperm membrane. Likewise, there are changes of the phosphorylation state of proteins within the sperm head (Lewis & Aitken 2001) that are correlated with the ability of the sperm to undergo acrosome reaction. Similarly, there is also a reorganisation of the acrosome with, for example, redistribution and modification of the activity of some of its contents (Yoshinaga & Toshimori 2003). There is considerable remodelling of the lipids of the sperm membrane (Jones 1998b), with a net loss of cholesterol and a considerable, but selective, loss of phospholipids (Hammerstedt & Parkes 1987), mainly from the inner leaflet of the membrane bilayer. There is also uptake of secreted glycoproteins (Tulsiani et al. 1998) and remodelling of these and endogenous glycoproteins within the sperm membrane. It appears that the addition of glycoproteins to the cell surface results in an increase in negative charge, which has, in turn, been suggested to contribute to the capacitation and egg-binding processes. Furthermore, glycosylation has been suggested as a means by which the sperm avoids immune detection in the female tract (Baker 2016). These changes in the membrane are associated with the acquisition of motility and fertilising ability (Jones 1998b).The acquisition of motility and fertilising ability are, however, not synonymous, although motility is required for fertility (Robaire et al. 2006). Acquisition of motility may also require modification to the dynein ATPase, together with the uptake of motility proteins and modifications to the intracellular compartmentalisation of calcium (Dorman & Dulcibella 2006).

Gervasi and Visconti (2017) summarised the changes that occur in the epididymis as follows: (i) in the caput epididymis, sperm are immotile, unable to fertilise, and the cytoplasmic droplet is close to the sperm head; (ii) there are low levels of sialic acid residues, few disulphide bonds, a high cholesterol to phospholipid ratio, and low membrane fluidity; (iii) the egg-binding protein, IZUMO1, which must be phosphorylated for fusion with the egg to occur, is not phosphorylated; (iv) in the cauda epididymis, the sperm are motile, able to fertilise and have either lost the cytoplasmic droplet or it has translocated to the distal end of the midpiece; (v) the

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sperm have high levels of sialic acid and disulphide bonds, a low cholesterol to phospholipid ratio, and high membrane fluidity. IZUMO1 in the equatorial segment of the sperm head is highly phosphorylated.

Physiology of the Accessory Glands

The accessory sex glands (vesicular gland, prostate, ampulla, and bulbourethral gland; Table 2.1) all have a critical requirement for androgens for their function. Testosterone is the prohormone for the more active androgen, dihydrotestosterone, which is produced by 5α–reductase in the accessory glands.

Ampullae are present as dilations of the terminal portion of the ductus deferens, just before they enter the pelvic urethra, where their main function is to act as reservoirs of sperm. In the bull, ram, and dog the ampullary glands, which are present, contribute slightly to the seminal plasma, whereas their contribution to the ejaculate is relatively more important in the stallion. The main constituent of ampullary secretion in the stallion is ergothioneine (Mann et al. 1956).

Vesicular glands are prominent in ruminants, the stallion, and the boar. They are sac-like in the stallion and boar and are firm, lobulated structures in the ram and bull. The glands are adjacent to the neck of the bladder and lateral to the ampullae. They open into the urethra just distally to the ductus deferens. Their secretion, which is generally watery, contributes substantially to the volume of the semen. In all species its secretion contains large quantities of citrate, although in the ruminants it also contains fructose and in the boar inositol (Marley et al. 1977).

The prostate is intimately related to the pelvic urethra and, in most species, is in two parts: its body surrounds the neck of the bladder, and its disseminated part spreads around the pelvic urethra, into which it has several openings. In the dog the prostate is the main accessory gland and is relatively large, forming a discrete organ around the urethra. The prostatic secretion is watery and contains large quantities of chloride ions, but neither citrate nor fructose nor inositol is present in high concentration (Huggins 1945).

In the stallion, bull, and ram the bulbourethral (or Cowper's) glands are small, rounded structures lying between the anus and urethra. Their watery secretion is discharged before coitus and is considered to cleanse the urethra of urine. In the boar the bulbourethral glands are large, cylindrical structures lying along each side of the intrapelvic urethra. In this species their secretion is very viscid because of its high sialomucin concentration (Boursnell et al. 1970) and combines with the secretion of the vesicular glands to produce a gelatinous phase of the seminal plasma (Boursnell & Butler 1973).

The physiological functions of the various constituents of seminal plasma remain a matter of debate (for a review, see Poiani 2006). There is much interspecies variation in the composition of seminal plasma, so it has been difficult to ascribe absolute functions to many of its constituents. Provision of energy, maintenance of osmotic pressure, chelation of free calcium ions, and buffering are some of the suggested functions; other possibilities include immunosuppression in the female genital tract and regulation of spermatozoan motility. Seminal plasma is also responsible for the coagulation of semen, which occurs shortly after ejaculation in many species. On the other hand, it has been argued that the wide variety in composition of seminal plasma between species indicates that it has no critical role, an argument that is emphasised by the ability of sperm to survive in relatively simple media that bear little resemblance to seminal plasma. Paradoxically, the seminal plasma of many species appears to contain spermicidal factors, especially in the postsperm-rich fraction of the species that produce a fractionated ejaculate.

The Penis

Erection

The penis comprises three tracts of erectile tissue and the penile urethra. The urethra is surrounded by the corpus spongiosum penis (CSP), which arises at the bulb of the penis and terminates in the glans penis. The erectile tissue of the glans penis is contiguous with the CSP, although the structure of the CSP glandis differs from that of the shaft of the penis. The dorsum of the penis is made up of the paired corpora cavernosa penis (CCP; Fig. 2.13), which arises in the two crura (roots) of the penis**,** and terminates behind the glans. The blood supply of all three tracts is via branches of the pudendal artery, but the venous drainage of the CCP is markedly different from that of the CSP. The CCP is drained via the root of the penis into the pudendal vein, whereas the CSP drains into the dorsal vein of the penis from its distal extremity (Ashdown & Gilanpour 1974). Thus both the supply and drainage of blood to the CCP is via the root of the penis, whereas the supply of the CSP is through the bulb and its drainage from its distal part.

Erection is achieved by raising the haemostatic pressure within the erectile tissues of the penis. It is mediated via a reflex that is predominantly coordinated at the spinal level (Andersson 2003) but is initiated and modulated centrally (Andersson & Wagner 1995). Most studies on the physiology of erection have been undertaken on the human, laboratory rodents, the dog, and, as it responds similarly to human penile smooth muscle, the bovine retractor penis muscle. In these species, erection is initiated by relaxation of smooth muscle within the CCP, leading to reduced vascular resistance and increased blood flow (Andersson & Wagner 1995). The penis is normally maintained in a flaccid state through tonic noradrenaline (norepinephrine) release from its sympathetic innervation, which causes contraction of the smooth muscle of the penile artery and CCP (Simonsen et al. 2002) through α_1–adrenoceptors and calcium-mediated pathways (de Tejada et al. 2004). The sympathetic stimulus also has antivasodilatory effects.

Erection is initiated via the parasympathetic system derived from the sacral plexus. Nitric oxide, produced from the action of parasympathetic–neural nitric oxide synthetase (nNOS) on L-arginine (Andersson & Wagner 1995), raises concentrations of cyclic guanosine monophosphate (GMP) in smooth muscle cells, leading to a reduced responsiveness to calcium and hence relaxation (Andersson 2003, de Tejada et al. 2004). Cyclic AMP-mediated pathways are also involved in vasodilation, in response to vasoactive intestinal peptide (VIP), prostaglandins E_1 and E_2, and noradrenaline (norepinephrine) acting through α_2–adrenoceptors (Simonsen et al. 2002). Increased flow through the penile arteries stimulates the endothelium to further release of these vasoactive substances, leading to further increases in blood flow (Kunelius et al. 1997). The role of each of these agents in other domestic species is not fully established. For example, although the bovine retractor penis muscle expresses both nNOS and VIP, their distribution is different from that in the penile smooth muscle of rodents (Vanhatalo et al. 2000).

Central nervous control of erection in primates and rodents is primarily derived from the hypothalamus and limbic system, with

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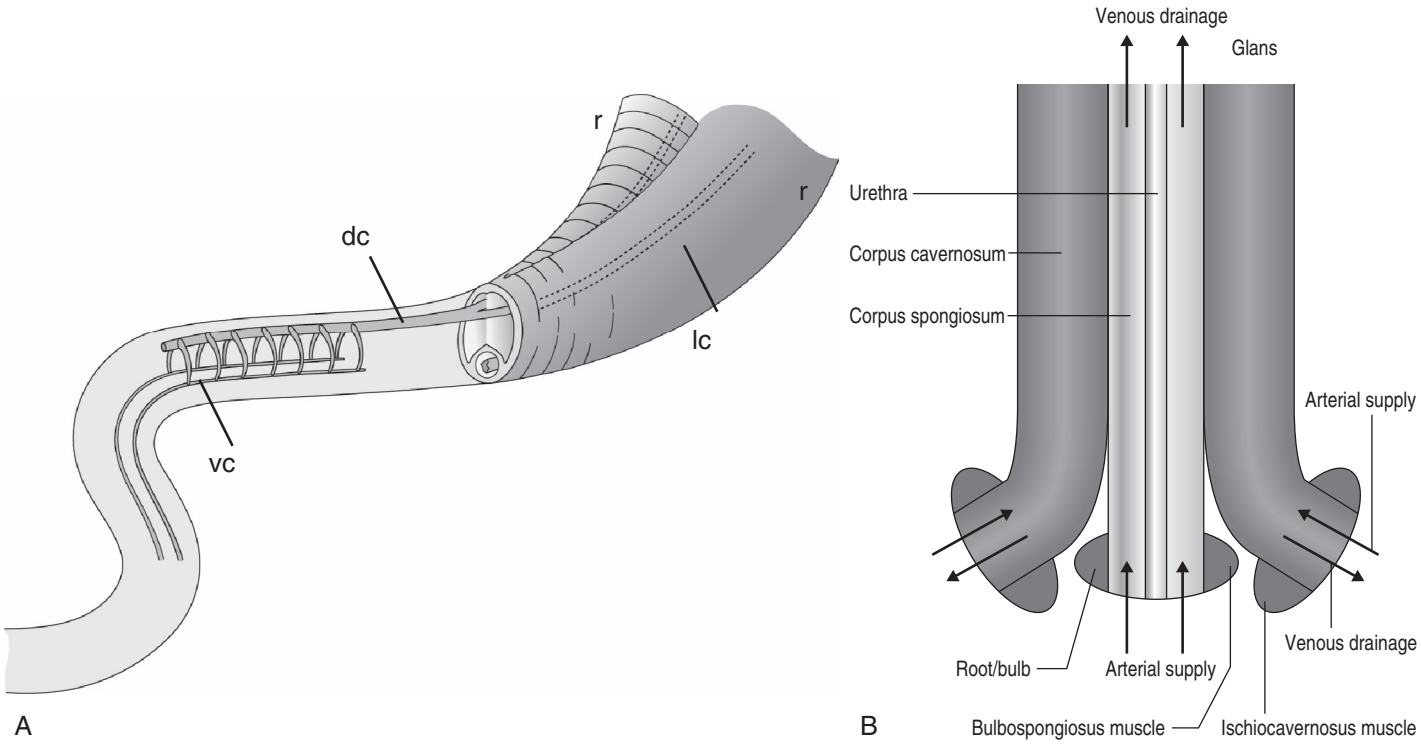

• **Fig. 2.13** Functional vascular anatomy of the bovine penis: (A) representational and (B) diagrammatic. Blood enters and leaves the corpus cavernosum via the arteries and veins of the crura (roots) of the penis, so that contraction of the overlying ischiocavernosus muscles occludes the venous drainage and forces blood into the penis under pressure. The blood passes through the penis in longitudinal canals *(lc).* Two canals arise in the roots *(r)* of the penis and unite to form a single dorsal longitudinal canal *(dc).* This gives off a series of lateral branches in the region of the sigmoid flexure, which unite to form paired ventral canals *(vc).* The corpus spongiosum is drained from its distal end, so contraction of the bulbospongiosus muscle only produces a transient increase in hydrostatic pressure. This is of sufficient magnitude to temporarily occlude the urethra, so that a bolus of semen can be propelled along its length. During detumescence vascular pressure is lost, and the penis is returned to the preputial cavity by the retractor penis muscle. (Redrawn and adapted with permission from Laing et al. 1988.)

the medial preoptic hypothalamic centre having a key integrating role (O'Donnell et al. 2006). Coordination of erection with other components of sexual responses appears to be an important role of the paraventricular nucleus (Swanson & Sawhenko 1983). The primary stimulatory pathways are mediated by oxytocin, with upstream regulation by the excitatory amino acids (Melis et al. 2004), glutamic and aspartic acids (and their agonists), and dopaminergic pathways (Andersson & Wagner 1995). Nitric oxide also stimulates the activity of central oxytocic stimulatory pathways. Inhibitory pathways include those producing gamma-amino butyric acid (GABA), serotonin, and opioids (Argiolas & Melis 2005).

Control of the processes of erection and ejaculation at the spinal level are dependent on a number of tracts of neural tissues within the spinal cord. First, there is the spinal nucleus of the bulbocavernosus (SNB: 'nucleus of Onuf'), a group of neurons in the low lumbar or sacral spine which regulate micturition, defecation, and the muscular contraction of ejaculation (Sakamoto 2011). In dogs, cats, and rodents (and humans) it is in the ventral horn, but in the pig it is dorsolateral from the central canal (Blok & Holstege 1998). It is sexually dimorphic, containing more neurons in males than females, a differentiation which occurs late in gestation under the control of androgens. Secondly, there is a group of lumbar spinothalamic neurons in the midlumbar spinal cord, which has been proposed (at least in the rat) as a 'spinal ejaculation generator', as it is activated during ejaculation – but not during other components of male behaviour (Truitt et al. 2003). Finally, there are projections of spinal gastrin-releasing peptide neurons from the midlumbar region to the sacral parasympathetic nucleus and the SNB, which are androgen controlled and which appear to regulate the erection and ejaculation reflexes.

Testosterone has a key role in the central and peripheral modulation of erectile function (Foresta et al. 2004), with a mode of action that is permissive rather than critical. In humans, testosterone may directly affect erection by acting directly as a vasodilator of the penile arterioles and cavernous sinusoid (Mikhail 2006), by inhibiting the breakdown of cyclic GMP or by regulating the synthesis of nitric oxide (Morelli et al. 2005). Testosterone is also responsible for the centrally mediated sexual behavioural responses.

Contraction of the ischiocavernosus muscles at the roots of the penis increases blood flow in the penile vasculature and occludes the veins that drain the CCP. In the dog the levator ani, the coccygeus, and the internal obturator muscles also participate in venous occlusion (Ninomiya et al. 1989). Small venules that drain the CCP of the human penis are occluded as a result of the lengthening that occurs during erection (Udelson et al. 2000). Despite these various mechanisms, venous occlusion is not complete as, when the dorsal vein of the penis is occluded, some drainage continues through smaller channels (Carati et al. 1988, Ninomiya et al. 1989). In humans venous drainage also continues during erection and, if excessive, can lead to erection failure (Hsieh et al. 2005). As blood

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flow in the penile arteries and CCP increases and as the venous drainage is interrupted, the cavernous spaces of the erectile tissue in the blind-ending CCP become engorged with blood, causing stiffening and lengthening of the penis (Beckett et al. 1974). At the peak of erection in the stallion, blood ceases to be able to enter the CCP from the pudendal artery because of the occlusion of the penile arteries by the action of the ischiocavernosus muscles (Bartels et al. 1984).

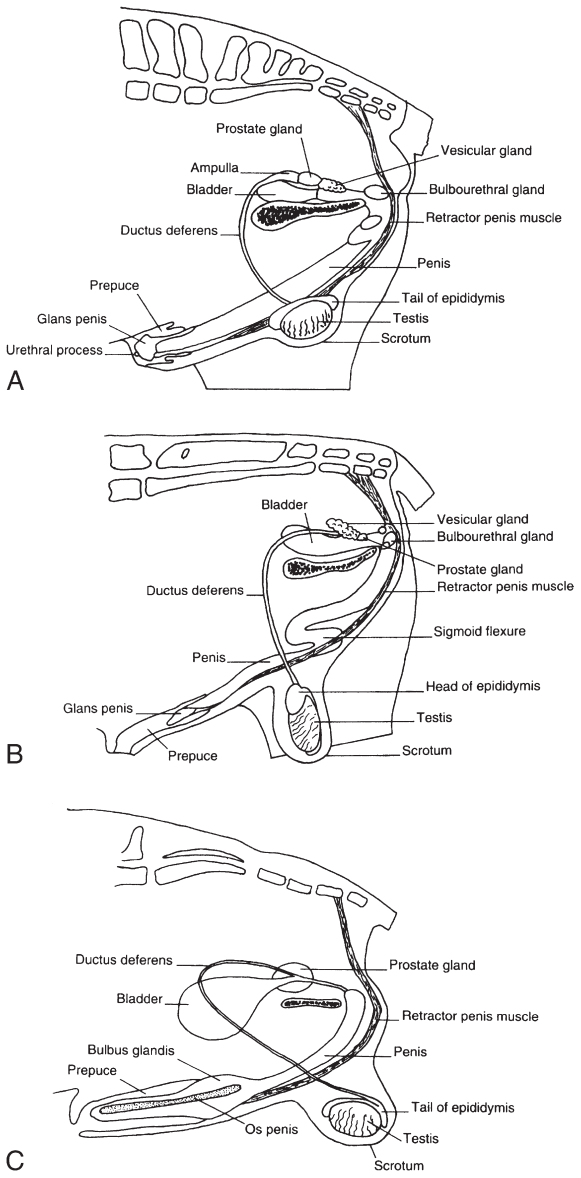
Ejaculation

The ejaculation reflex is stimulated by sensory nerves within the glans penis, which transmit to the spinal cord through the dorsal nerve of the penis, a branch of the pudendal nerve (Johnson & Halata 1991). The integrity of this nerve is essential for the ejaculation reflex to take place, and if it becomes damaged, ejaculation, though not erection, becomes impossible (Beckett et al. 1978). Pressure, tactile sensation, and, in the bull, temperature are the main stimulants to ejaculation. Afferent fibres also pass along the hypogastric nerve into the spinal cord via the sympathetic chain (Baron & Janig 1991). Efferent fibres are derived from the sympathetic and parasympathetic outflows, whereas somatic motor fibres innervate the bulbospongiosus muscle (Giuliano & Clement 2005). Ejaculation is primarily coordinated as a spinal reflex in the lower lumbar and sacral segments of the spinal cord, although with central regulation via the medial preoptic area of the hypothalamus and connecting nuclei. Central control of ejaculation is facilitated by dopamine and inhibited by serotonin (Hull et al. 1995, Waldinger & Olivier 2004). There are two components to ejaculation: (1) emission and accessory gland secretion, which are primarily controlled by the autonomic nervous system; and (2) propulsion, which is controlled via somatic nerves. Emission is under the primary control of sympathetic noradrenaline (norepinephrine). Propulsion of semen out of the urethra is caused by rhythmic contractions of the bulbospongiosus muscle, which overlies the CSP in the bulb of the penis, and of smooth muscles of the urethra (Giuliano & Clement 2005). This is accompanied by proximal closure of the bladder neck and intermittent relaxation of the external urethral sphincter and urogenital diaphragm.

Contraction of the bulbospongiosus muscle causes increased haemostatic pressure in the CSP. However, because the CSP drains from its distal end, the high pressures achieved in the CCP cannot be attained. Thus each contraction of the bulbospongiosus muscle causes a transient wave of increased pressure in the CSP, which progresses from the bulb to the glans, where it dissipates by dorsal venous drainage of the blood. Because the CCP is turgid, the increased pressure within the CSP causes a wave of occlusion of the urethra. This progressive wave of urethral occlusion, assisted by the contraction of the muscle that surrounds the extrapelvic urethra, causes conduction of boluses of semen along the urethra.

Species Differences

Details of the anatomy of the penis vary greatly between species, and, as a result, details of the functional anatomy of erection are similarly variable. In Artiodactyla (Fig. 2.14), the penis has a thick, fibrous tunica albuginea overlying the CCP and surrounding the urethra, and the individual cavernous spaces within the CCP are relatively small. The penis also has a sigmoid flexure, which is either caudal (postscrotal), as in the ruminants, or cranial (prescrotal) to the scrotum, as in the boar. In these species, relatively little blood enters the penis during erection, although the blood pressures achieved



• **Fig. 2.14** Comparative anatomy of the penis of (A) the stallion, (B) bull, and (C) dog. (Redrawn and adapted with permission from Laing et al. 1988.)

are considerable, exceeding 40,000 mm Hg during ejaculation. In order that the increased pressure induced by ischiocavernosus muscle activity can be transmitted throughout the length of the penis, specialised artery-like vascular canals and the longitudinal canals run the length of the penis. Paired canals arise in the crura of the penis, which fuse shortly afterwards to produce a single dorsal canal. Thereafter, either side of the dorsal canal gives rise to a series of branches, which join together to form paired ventral canals. The dorsal canal runs for the proximal third of the penis and the ventral canals for the remaining distance, with a short distance of overlap with the dorsal canal. Lengthening of the penis is achieved partly by longitudinal expansion of the cavernous spaces between the trabeculae of the CCP, but mainly by straightening of the sigmoid flexure of the penis (Ashdown 1970). As a result, the penis, which is normally carried high in the preputial cavity, is fully exteriorised from the narrow preputial orifice. Obliteration of the sigmoid flexure and forward movement of the penis

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is made possible by the very loose arrangement of the connective tissue that surrounds the penis and prepuce. In the ruminants full erection is only briefly attained during the single ejaculatory thrust, but copulation is more prolonged in the boar. Erection is terminated by cessation of ischiocavernosus muscular contraction, and the penis is returned to the preputial cavity by contraction of the retractor penis muscles, which restores the sigmoid flexure.

The other domestic species have a musculocavernous penis, in which the tunica albuginea is less pronounced and the cavernous spaces of the erectile tissue are larger than in the artiodactyls. In the stallion (Nickel et al. 1973, Amann 1993) tracts of longitudinally oriented smooth muscle fibres are associated with the trabeculae of the CCP. These are normally in a state of tonic contraction, holding the penis in the prepuce. The tone in these muscles falls during erection and micturition, leading to prolapse of the penis from the prepuce. In the stallion and dog (Evans & deLahunta 1988) erection produces increases in both length and girth of the penis and, as there is no sigmoid flexure, the lengthening of the penis is caused entirely by vascular engorgement.

During ejaculation, the glans of the penis of the bull and goat becomes coiled (Ashdown & Smith 1969), whereas the vermiform appendage of the penis of the ram shows a vigorous flicking movement. It is probable that these conformational changes are to assist with deposition of semen in and around the external os of the cervix. The glans penis of the stallion and boar engages in the cervical canal, with ejaculation occurring through the cervix into the uterine lumen. Thus the penis of the boar adopts a spiral conformation during copulation, which mirrors that of the cervix of the sow, but the stallion shows only a very pronounced enlargement of the glans penis during ejaculation. The dog is unique among the domestic animals in having a bone in the penis (os penis), in whose grooved ventral floor the urethra lies. During copulation the penis of the dog is gripped by the levator vestibuli of the vagina of the bitch, whereupon engorgement of the bulbus glandis occurs. Ejaculation occurs over a prolonged period of time, with the brief production of the preejaculatory and sperm-rich fractions, followed by a very protracted deposition of prostatic fluid during the copulatory tie.

Development of the Penis

The development of the external genitalia of the male was summarised by Pansky (1982). In brief, initial development of the genital organs is similar in both sexes, beginning with the development of the cloacal folds, which fuse together in front of the cloacal membrane to form the cloacal eminence. Thereafter, the cloacal membrane ruptures to form the urogenital and anal openings, the cloacal folds form the genital folds, and the cloacal eminence elongates to form the genital tubercle. Concurrently, a second pair of folds, the labioscrotal folds, develop laterally to the genital folds. At this stage the sexes are similar, but thereafter there is androgen-dependent development of the genital tubercle of the male to form the phallus. The phallus pulls the urogenital folds forward, such that the folds elongate along its ventral side to form the lateral walls of the urethral groove. The urethral groove progressively fuses along its length to form the definitive urethra, while the opening of the urethra moves distally towards the glans. The erectile tissue of the CCP and CSP differentiate at the same time. Finally, the labioscrotal folds grow towards each other and fuse in the midline to form the scrotum. Androgens are required for the differentiation of the male external genitalia; mutations of any step in the androgen synthesis pathway or in the activity of androgen receptors result in abnormalities or failure of the male genitalia to develop.

At birth the penis is fused with the prepuce throughout its length, with small lateral veins draining the erectile tissue. During prepubertal development the connective tissue joining penis and prepuce breaks down and the veins become occluded. The frenulum is the most substantial of the connections between the penis and prepuce, frequently containing quite large blood vessels. It is often the last part to break down and may not infrequently persist into postpubertal life.

Libido and Mating Behaviour

Libido is primarily dependent on androgenic steroid hormones, which allow mating and aggressive behaviour to occur, as well as maintaining the function of all parts of the male reproductive system. Libido is seldom expressed in animals that are castrated before puberty, although if a mature animal that has learnt to copulate is castrated, erection and copulation may persist for long periods or, occasionally, indefinitely. Despite the dependency of male behaviour upon androgen, there has been much debate over the relationship between absolute concentrations of androgen and libido (Foote et al. 1976, Wodzicka-Tomaszewska et al. 1981). Some have argued a permissive role for androgen, whereas others have demonstrated positive correlations between testosterone concentrations and measures of libido. Breeds of bull that are aggressive and respond quickly to the presence of an oestrous female tend to have higher testosterone concentrations than the more phlegmatic breeds, but whether this is a causal relationship remains unclear.

The males of those domestic species that are naturally herd animals spend a great deal of time detecting oestrus (Chenoweth 1981). Oestrous females of many species secrete pheromones to attract males, whereas others, notably the cow and some breeds of pig exhibit homosexual behaviour as a signal to the male of the presence of oestrus. All males smell the perineal region of the female, and the odour of the oestrous female induces the so-called 'flehmen' reaction in the bull, ram, camel, and stallion – a characteristic raised posture of the head and elevation of the upper lip. Females that are not in oestrus signal their objection to the advances of the male and, at the least, will respond by moving away or perhaps by attacking the male in the offensive manner peculiar to the species. During prooestrus the interest of the male in the female is increased, but, whereas she associates with the male, she will not permit mounting. Oestrous females signal receptivity by squatting, urinating, moving the tail to one side, and remaining stationary (Fig. 2.15). During this foreplay, the male becomes progressively aroused; there are frequent erections of the penis, with emission of accessory fluid, and many unsuccessful attempts to mount the female. Finally, mounting and copulation occur.

Stallion

After intromission the stallion performs a succession of copulatory movements of the hindquarters, which, within a minute, culminate in ejaculation. During ejaculation, successive waves of urethral peristalsis can be palpated on the lower surface of the penis, while the stallion exhibits a characteristic 'flagging' movement of the tail (Fig. 2.16). The stallion then dismounts.

Ruminants

Copulation in all the domestic species of ruminants is brief. After detecting an oestrous female, mounting is followed quickly by the single ejaculatory thrust. The male then immediately dismounts

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• **Fig. 2.15** Signs of oestrus in the mare include (A) protrusion of the clitoris and (B) squatting, urination and moving the tail to one side. (Photographs courtesy Dr. Jonathan Pycock.)

• **Fig. 2.16** The main external sign of ejaculation in the stallion is tail flagging. Peristaltic waves can also be palpated in the penile urethra.

but frequent repeated matings occur. Farm management of rams and bulls should take this high mating frequency into account. Thus whereas individual sires can service quite large groups of spontaneously cyclic females, the groups of females have to be much reduced if these have undergone oestrous synchronisation (see Chapters 8, 25 and 26). Furthermore, care has to be exercised in the use of young sires. The testis does not reach its full size and sperm-producing capacity until 1 to 2 years after puberty, nor does the epididymis reach its full length until the same time (reviewed by Salisbury et al. 1978). Until maturity has been reached, groups of females should be smaller than for adult animals. If overused, both the numbers of sperm produced are too few for adequate conception rates to be achieved and sperm from the tail of the epididymis are ejaculated before their functional maturation is complete. Thus sperm numbers and sperm maturity are both adversely affected, causing severe limitation of the chances of conception.

Boar

Copulation is relatively prolonged in pigs, lasting for between 5 and 15 minutes. After intromission the first phase is occupied by a series of vigorous thrusting movements of the hindquarters of the boar. During this phase the first part of the fractionated ejaculate is produced, which comprises only accessory fluid. The second phase is quieter and accompanied by production of the sperm-rich fraction of the ejaculate. This is followed by a final, more vigorous phase, in which the third, jelly-like accessory secretion is discharged. Ejaculation occurs directly into the uterus, which is distended with semen immediately after copulation, whereas the cervix is often sealed with a gelatinous plug. The large volume of semen produced by the boar is necessary to assist the transport of its spermatozoa along the extensive length of the uterus of the sow.

• **Fig. 2.17** The copulatory tie in the dog.

Dog

The dog achieves intromission by vigorous thrusting of the hindquarters. Once intromission has been achieved, the bulbus glandis swells considerably, while the constrictor vulvae muscles of the bitch contract behind it, thus forming the 'copulatory tie'. The sperm-rich fraction of canine semen is ejaculated within as little as 80 seconds of intromission, so that conception may occur even if copulation does not proceed through to its second stage. In this second stage the dog dismounts but remains 'connected' to, but faces away from, the bitch (Fig. 2.17). This change of position causes the penis to become bent through an angle of 180 degrees; the efferent veins of the penis are thereby occluded and the penis remains turgid. The function of the tie may be to prevent penile detumescence throughout the prolonged second stage of copulation, during which some 30 mL of sperm-free accessory fluid is pumped into the vagina and then to the uterus, where it flushes the sperm-rich fraction through the uterus. The sexes remain locked until the vulval muscles relax and penile erection wanes.

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Cat

During mating, the tom mounts the queen and grasps her neck with his teeth. As the tom adjusts his position the queen paddles her hind legs, continuing to do so at an increasing frequency during the 10 seconds, or so, for which coitus lasts. The queen cries out during copulation, and, as the tom dismounts, she may strike out at him, displaying the typical 'rage' reaction. This is followed by a period of frantic rolling and licking at the vulva. As soon as the postcoital reaction has ceased, the tom will attempt to mount again. Several matings may therefore occur within the first 30 to 60 minutes. The cat is an induced ovulator (see Ch. 1; Shille et al. 1983), so the number and frequency of matings are important in ensuring that the LH surge is of sufficient magnitude to cause ovulation (Tsutsui & Stabenfeldt 1993).

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3

Puberty and Seasonality

RICHARD LEA AND GARY C. W. ENGLAND

In nature it is the general rule that animals breed once annually, and parturition occurs in the spring, the time most favourable to the progeny because the early neonatal period of their life will occur during the period of increasing light and warmth and at the time when food for the mother is most abundant to ensure adequate lactation. Under the conditions of feeding and housing provided by domestication, the breeding season tends to be lengthened, and some of our species, particularly cattle, may breed at any time during the year; all domesticated animals, however, show a constant tendency to revert to the natural breeding season, as evidenced by reduced fertility during summer and early autumn in sows.

Puberty and the Onset of Cyclic Activity

The young female animal shows no evidence of recurring or cyclic periods of sexual receptivity. The onset of such changes, when the female becomes sexually mature and able to reproduce**,** is referred to as puberty. Among females of the domestic species, puberty precedes the development of physical maturity and, although they become capable of reproducing, their efficiency, particularly with respect to their fecundity (number of offspring), has not reached its maximum. For example, juvenile ewes mated just after puberty produce on average 0.6 lambs to weaning compared with 1.2 lambs produced from ewes 1 year older (Kenyon et al. 2004). This is reported to be caused by a lower ovulation rate, increased fertilisation failure, embryo loss and failure to be mated by the ram (Edwards et al. 2016).

The initiation of puberty is largely a function of the animal's age and maturity because the female is born with a genetic potential for cyclic reproductive activity. Provided the environmental influences are favourable at this time, then once the 'biological clock' is started, it will continue for as long as the environment remains promising. In none of our domestic species is there a physiological change comparable with the menopause of women. However, reproductive senescence is reported in killer whales and pilot whales, and in these species, this is thought to be a strategy to boost the ecological fitness. Postreproduction aged females take a leadership role in the group and are thus central to the maturity of the younger members of the pod (Brent et al. 2015).

Among nonseasonal polycyclic animals such as the cow and sow, the recurring cyclic activity is interrupted by pregnancy, lactation and pathological conditions. In those species that are seasonally polycyclic (i.e., the mare, ewe, doe (or nanny) goat and cat) or monocyclic (i.e., the bitch) there are periods of sexual quiescence or anoestrus.

When the female reaches puberty, the genital organs increase in size. During the prepubertal period the growth of the genital organs is very similar to that of other organ systems. At puberty their growth rate is accelerated, a point well illustrated in the gilt in which the mean length of the uterine horns is increased by 58%, the mean weight of the uterus by 72% and the mean weight of the ovaries by 32% between 169 and 186 days of age (Lasley 1968). Females of domestic species reach the age of puberty at the following times:

- Mare: 1 to 2 years
- Cow: 7 to 18 months
- Ewe: 6 to 15 months
- Doe or nanny goat: 4 to 8 months
- Sow: 6 to 8 months
- Bitch: 6 to 20 months
- Queen cat: 7 to 12 months

The changes that occur at puberty depend directly upon the activity of the ovaries, which have two functions: the production of the female gametes and the synthesis of hormones. Consider the changes that occur in the ovary of the heifer calf. At birth the ovaries may contain up to 150,000 primary or primordial follicles apiece, and each follicle consists of an oocyte surrounded by a single layer of granulosa cells but no thecal cells. Soon after birth, the ovaries start to produce growing follicles, which consist of an oocyte with two or more layers of granulosa cells and a basement membrane. The stimulus for the development of these follicles is intraovarian; until the heifer reaches the age of puberty, they will develop only to the stage in which they have a theca interna and then start to undergo atresia. Further development of these follicles to produce mature Graafian or antral follicles, of which there are about 200 growing follicles at puberty, is dependent upon the stimulus of gonadotrophic hormones. Despite the absence of oestrous cycles, there is follicular growth, as has been shown using transrectal ultrasonography in calves from 2 weeks of age. It was noted that there were follicular waves in response to follicle-stimulating hormone (FSH) secretion that were similar to those of the adult and that individual follicular development was characterised by growing, static and regressing phases (Adams et al. 1994).

The sheep has been used extensively for studying many of the mechanisms involved in the initiation of puberty; however, it must be stressed that seasonality exerts an overriding influence in this species. The onset of puberty is signalled either by the occurrence of the first oestrus or the first ovulation; in the ewe lamb these do not occur simultaneously because the first ovulation is not preceded by behavioural oestrus. A similar response is

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seen in sexually mature ewes at the onset of the normal breeding season.

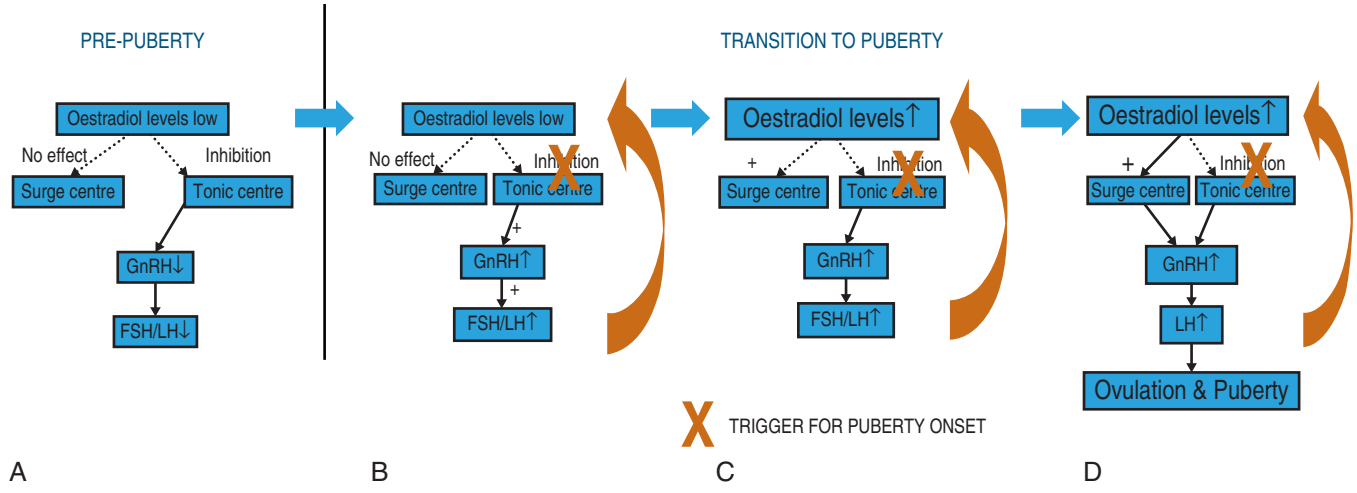
The hormone that is primarily associated with the onset of ovarian activity, and hence puberty, is luteinising hormone (LH). In adult ewes during the normal breeding season, basal LH concentrations increase together with the LH pulse frequency to one per hour during the period of maximum follicular growth. This results in the development of follicles to the preovulatory stage and to their secretion of oestradiol, which activates the LH surge causing ovulation and corpus luteum formation. In the prepubertal ewe lamb, LH pulses occur at similar amplitudes but much lower frequencies (one every 2–3 hours). As a consequence, follicular growth is insufficient to activate the LH surge necessary for final follicular maturation and ovulation.

Experimental evidence in prepubertal ewe lambs has shown that ovarian follicles are capable of responding to exogenous gonadotrophin stimulation, and the pituitary is capable of secreting LH at a frequency to stimulate ovulation. The failure of the prepubertal ewe lamb to undergo ovulation and exhibit oestrus is caused by the high threshold for the positive feedback effect of oestradiol, and thus there is no LH surge. Indeed, the endocrine trigger for puberty is based on sex steroid feedback regulatory mechanisms, which operate at the level of the tonic or surge centres of the hypothalamus (Day et al. 1984, Ebling et al. 1990). The sequence of events that results in the onset of puberty is depicted in Fig. 3.1. Puberty onset relies on the secretion of ovarian oestradiol so that the circulating concentration is sufficient to stimulate a preovulatory gonadotrophin-releasing hormone (GnRH) and LH surge. Before puberty, when the concentration of oestrogen is too low to exceed the necessary threshold required for ovulation, the oestrogen that is present has an inhibitory effect on the tonic centre of the hypothalamus (Fig. 3.1A). As puberty approaches, the inhibitory effect of oestrogen on the tonic centre is reduced (Fig. 3.1B). Consequently, the hypothalamic-pituitary-gonadal axis (HPG) axis starts to become more active so that the pulsatile frequency of GnRH increases as do FSH and LH. The increased FSH thus stimulates more follicle development, which then starts secreting oestrogen. This process continues until oestrogen concentrations are high enough to stimulate the surge centre of the hypothalamus, thus causing a preovulatory GnRH and LH surge (Fig. 3.1 C, D). The endocrine trigger for puberty is therefore considered to be the loss of feedback inhibition of oestrogen on the tonic centre of the hypothalamus (Ebling et al. 1990).

Male Puberty Onset

Different parameters can be used to define puberty onset in the male. These may be behavioural or relate to the age of first ejaculation, both of which occur before sperm numbers are high enough to achieve fertilisation. Indeed, younger males will have smaller testes and relatively short epididymides, and therefore daily sperm production will be lower than in more mature animals. Consequently, males that are within a few months of puberty will have a reduced capacity to establish high pregnancy rates. The quality of the ejaculate may therefore also be considered as an index of puberty, in terms of sperm production and the generation of sufficient numbers, to reliably establish a pregnancy.

Similar to the female, puberty in the male occurs as a result of a decrease in hypothalamic sensitivity to oestradiol and testosterone. Because the male testosterone crosses the blood–brain barrier and is then converted to oestradiol by the enzyme aromatase, the inhibitory effect is primarily caused by oestradiol. As puberty onset occurs in the male, the inhibitory effects of oestradiol and testosterone on hypothalamic GnRH secreting neurons are reduced (Fig. 3.2). The increased pulsatile release of GnRH then stimulates more LH and FSH, which stimulate the testis to undergo spermatogenesis, thus initiating the onset of puberty. In the male the rise in LH begins earlier than in the females. Consequently, the rise in testosterone is gradual, and in the sheep this includes the 2-month period required for spermatogenesis. It is thought that the earlier initiation of puberty in the male allows sperm development to occur before the females develop the capacity to ovulate (Foster et al. 2006).



• **Fig. 3.1** Puberty onset in the female. Before puberty: (A) The ovary does not secrete enough oestradiol to stimulate the surge centre of the hypothalamus to produce high amplitude episodes of GnRH. Low concentrations of oestradiol also have a negative feedback effect on the hypothalamic tonic centre. (B) As the transition to puberty begins, the sensitivity of the tonic centre to oestradiol inhibition reduces, enabling hypothalamic neurons to secrete GnRH. (C) Consequently, the frequency of LH and FSH episodes increase, which stimulates the ovary to produce more oestradiol. (D) When the concentration of oestradiol reaches a threshold, it stimulates the surge centre to secrete a preovulatory surge in GnRH, which results in a preovulatory surge in LH, ovulation and the initiation of puberty.

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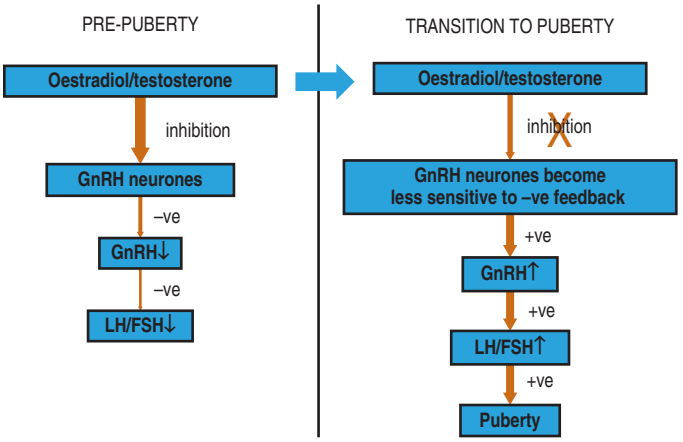

• **Fig. 3.2** Puberty onset in the male. Before puberty, low concentration testosterone crosses the blood–brain barrier and is converted to oestradiol by aromatase. Both oestradiol and testosterone have a negative feedback effect on hypothalamic GnRH neurons. As the transition to puberty begins, the sensitivity of the hypothalamic neurons to oestradiol reduces. Increased GnRH release stimulates LH and FSH secretion, which act on the testis to initiate puberty.

Seasonal Breeding as a Recurring Model of Puberty

In those species that are seasonal breeders such as the ewe, mare and queen, the age at which puberty occurs will be influenced by the effect of season of the year. For instance, a filly born early in the year (i.e., January or February) may have her first oestrus in the May or June of the following year (i.e., when she is 16 or 17 months old). A filly foal born late in the year, July or August, may not have her first oestrus until she is 21 or 22 months old.

By definition, seasonal breeders undergo a period of anoestrus (nonbreeding season), and the annual transition to the breeding season has many similarities to puberty. In both cases, the fundamental endocrine mechanisms that enable a sexually quiescent animal to become sexually active are very similar. For example, the anoestrous and prepubertal animal is characterised by the lack of an LH surge necessary for ovulation to occur, and yet in both cases the ability to produce an LH surge in response to a stimulus (GnRH) is present. In both cases, LH secretion is pulsatile but low frequency, and thus the stimulus for an adequate rise in oestrogen is missing. Finally, the trigger for puberty described previously also applies to animals transitioning between anoestrus and the breeding season.

Other factors are also involved. The frequency of LH secretion is dependent upon GnRH from the hypothalamus, which is controlled by an area in the hypothalamus referred to as the neural GnRH pulse generator. Age-related changes in brain morphology and neuronal cytoarchitecture may also be important because extrapolation from studies performed in rats, for example, has shown an increase in the number of GnRH cells with spine-like processes on the soma and dendrites. In addition, the inhibitory effect of opioid peptides on LH secretion is reduced with age, which may provide a neurochemical explanation for the changes in pituitary sensitivity to oestradiol feedback that occur at puberty (Bhanot and Wilkinson 1983, Wray and Hoffman 1986).

The reason for the 'silent' first oestrus of the pubertal animal is believed to be because the central nervous system requires priming with progesterone before it will respond and the animal will show behavioural signs of heat. The first ovulatory cycle has been shown to be short in pubertal heifers (7.7 ± 0.2 days), and the first corpus luteum (CL) not only has a shorter than normal life span but is also smaller in size. One explanation for this is that the dominant follicle, from which the first ovulation arises, has already entered the static phase of growth. The subsequent interovulatory interval is normal (Adams 1999). Once puberty has occurred, in most domestic species, there are waves of follicular growth and regression. However, even in the prepubertal animals, follicular waves have been identified; these have been recorded in heifers (Evans et al. 1994a, b) and fillies (Nogueira and Ginther 2000).

Melatonin, Puberty and Seasonality

Melatonin and Other Pineal Peptides

The pineal gland appears to have an important role in controlling reproduction in seasonal breeding species and in the timing of puberty by influencing the release of FSH, LH and prolactin. Although much of the interest has been in the action of the indoleamine melatonin, there is increasing interest in the other pineal peptide hormones, namely arginine vasotocin, gonadotrophin and prolactin-releasing and inhibitory hormones. There is some suggestion that melatonin may not act directly upon the hypothalamus/anterior pituitary, but indirectly via the other pineal peptide hormones.

Melatonin drives the reproductive response of the ewe to inductive photoperiods (Bittman et al. 1983). Rhythmic administration of melatonin to adult ewes exerts a similar effect to increased hours of darkness by inducing the onset of the breeding season (Arendt et al. 1983) and causes changes in prolactin concentrations in the plasma that are similar to those after exposure to short days (Kennaway et al. 1982). In sheep an intact pineal gland is required for a normal photoperiodic response to altered daylight patterns; however, other seasonal environmental cues are important because pinealectomised ewes still show seasonal breeding (Lincoln et al. 1989).

The mare is a seasonal breeder but is 'switched on' by increasing day length. The pineal gland is involved because if it is removed, the mare does not show a normal response to changes in photoperiod. In intact mares, melatonin concentrations increase during hours of darkness (Grubaugh et al. 1982). There is some evidence that foals are conditioned at an early age and develop a pattern of melatonin secretion from about 7 weeks of age (Kilmer et al. 1982).

The Role of Kisspeptin in the Onset of Puberty and Seasonality

For many years it was thought that an increase in the frequency and amplitude of GnRH secretion was the main marker of puberty. However, puberty and seasonality are influenced by a number of environmental factors, including nutrition and degree of fatness (discussed later in this chapter), and this is indicative of a higher level of control. This control is mediated by presynaptic neurons that are sensitive to environmental influences and, in turn, stimulate neuronal GnRH secretion. One of the key proteins released by presynaptic neurons is the hormone kisspeptin, which when released, binds to its receptor (KISS1R or GPR54: galanin-like G protein-coupled receptor 54), which is expressed on GnRH secreting neurons. There is now considerable evidence that an intact kisspeptin signalling pathway is a prerequisite for the onset of puberty in mammals, which has led to the expression that 'puberty begins with a Kiss' (Dungan et al. 2006, Terasawa et al. 2013).

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An addendum to the mechanism describing the onset of puberty (Figs 3.1 and 3.2) must therefore also include kisspeptin-secreting neurons that are found in the arcuate nucleus and the periventricular nucleus of the hypothalamus. The rising oestrogen concentrations that eventually activate the HPG axis do so by a positive feedback effect on kisspeptin signalling. Indeed, kisspeptin-secreting cells express the oestrogen receptor subtype alpha (ERα) necessary for the feedback effects of oestrogen, whereas the receptor is not present on GnRH secreting cells (Clarke et al. 2009). Similarly, it is thought that the negative feedback effect of oestrogen before puberty works through reduced kisspeptin levels. This goes beyond puberty because the effects of oestradiol on kisspeptinergic nuclei require integration into the mechanism of ovulation described in Chapter 1.

Kisspeptin also plays a key role in seasonality. Studies in sheep have shown that the expression of the kisspeptin gene increases with reduced photoperiod, and these seasonal changes occur in the arcuate nucleus of the hypothalamus. Furthermore, kisspeptin treatment of anoestrous sheep has been shown to restore reproductive function (Clarke et al. 2009). This raises the possibility of a future clinical use of kisspeptin in the manipulation of seasonality in this species.

Although the primary translation product of the kisspeptin gene is a 145 amino acid polypeptide, various isoforms are produced and a decapeptide (kisspeptin 10) has full biological activity central to the control of seasonality in both short- and long-day breeders (Revel et al., 2007). The most investigated long-day breeder is the Syrian hamster, and studies have shown that kisspeptin 10 administration can restore reproductive function under photoinhibitory conditions (Ansel et al. 2011). Similarly, in sheep, as short-day breeders, kisspeptin 10 can induce ovulation in anoestrous ewes (Caraty et al. 2007).

Despite these advancements, the mechanism by which reduced melatonin has opposite effects on seasonality in short- and long-day breeders and how this relates to kisspeptin function is not fully understood. However, some insight has come from the study of another family of peptides called RFamide-related peptides (RFRP) that are also expressed by hypothalamic neurons and have been shown to inhibit gonadotrophin release (Henningsen et al. 2016). RFRP3 appears to be the main inhibitory peptide in mammals and is thought to act on the same GnRH neurons that are regulated by kisspeptin 10. Therefore it has been postulated that seasonality is regulated through the relative activities of kisspeptin 10 and RFRP3 secreting neurons in the hypothalamus. Further research is required to truly clarify the precise mechanisms involved.

Other External Factors Influencing the Time of Onset of Puberty and Seasonality

The time of onset of puberty is determined by the individual's genotype, with smaller breeds of animal tending to be slightly more precocious. However, this inherent timing is influenced by a number of external factors.

Nutrition

There is good evidence that, in most domestic species, the onset of puberty is closely linked to the attainment of a critical body weight, as well as a minimum percentage body fat, or metabolic mass (Frisch 1984). Thus nutrition is an important factor. Animals that are well fed with good growth rates reach puberty before those that are poorly fed with slow growth rates. However, unless the animal is severely malnourished, cyclical activity will eventually occur. Although the cellular mechanisms which underlie metabolic influences on hypothalamic GnRH neurons are not fully characterised, dietary changes clearly alter GnRH secretion. For example, dietary restriction in sheep delays puberty by an effect on both frequency and amplitude of GnRH secretion (I'Anson et al. 2000).

The protein leptin (see later in this chapter), secreted by white fat cells in adipose tissue, is an important link between metabolic status and the neuroendocrine axis. Although several studies have shown that leptin treatment can advance the onset of puberty in both restricted and ad lib fed animals (Barash et al. 1996, Ahima et al. 1997) and that serum leptin concentrations increased in pigs (Qian et al. 1999) and heifers (Garcia et al. 2002) at puberty, it is generally accepted that leptin is not the triggering signal for puberty, but a permissive signal that enables puberty to occur (Barb and Kraeling 2004). Leptin acts as a metabolic 'gate' as circulating concentrations increase over the course of pubertal development; serum leptin levels reach a putative stimulatory threshold that permits activation of the hypothalamic–pituitary–gonadal axis. This is associated with a decrease in the negative feedback action of oestradiol on the hypothalamic–pituitary axis, and stimulation of adipose leptin gene expression (Barb and Kraeling 2004). Because GnRH neurons do not express leptin receptors, the effects of leptin on puberty must be indirect and are thought to be mediated by presynaptic hypothalamic neurons. Specifically, leptin is thought to act not only through kisspeptin neurons, but also through POMC (proopiomelanocortin) and neuropeptide Y (NPY) neural networks. In other words, these neurons are thought to transmit leptin signals to the neural networks that regulate GnRH secretion (Amsteldam and Williams 2014). Other metabolic factors, including glucose and fatty acids, may also act indirectly on GnRH through activities on 'metabolic sensitive' neurons, which then influence kisspeptin and ultimately GnRH secretion.

Proximity of the Male

Studies in sheep and pigs have shown that exposure to the male of the species will advance the timing of the onset of puberty. This so-called 'ram or boar effect' is probably mediated by pheromonal and other sensory cues influencing hypothalamic GnRH secretion.

Climate

Anthropomorphic extrapolation has assumed that animals living in the tropics reach puberty at an earlier age than those in temperate climates. Studies carried out in Zambia have shown that in cattle this is not true.

Disease

Any disease that can influence the growth rate, either directly or because of interference with feeding and utilisation of nutrients, will delay the onset of puberty.

Puberty and Seasonality: A Species Overview

Horse

Puberty

Fillies are often seen in oestrus during their second spring and summer (when they are yearlings); however, under natural

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conditions, it is unusual for them to foal until they are over 3 years old. As outlined previously, the timing of puberty will be affected by season, with fillies born earlier in the year having their first oestrus 5 months earlier than those born later in the year.

Seasonality

The mare is normally a seasonal breeder, with cyclic activity occurring from spring to autumn; during the winter she will normally become anoestrus. However, it has been observed that some mares, especially those of native pony breeds, cycle regularly throughout the year. This tendency can be enhanced if the mares are housed and given supplementary food when the weather is cold and inclement and if additional lighting is provided when the hours of daylight are short.

Horse breeding has been influenced by the demands of thoroughbred racing because, in the northern hemisphere, foals are aged from 1 January, irrespective of their actual birth date. As a result, the breeding season for mares has been, for over a century, determined by the authorities as running from 15 February to 1 July. Because the natural breeding season does not commence until about the middle of April and maximum ovarian activity is not reached until July, it is obvious that a large number of thoroughbred mares are bred at a time when their fertility is suboptimal.

Winter anoestrus is followed by a period of transition to regular cyclic activity. During this transition the duration of oestrus may be irregular or very long, sometimes more than a month. The manifestations of heat during the transitional phase are often atypical and make it difficult for the observer to be certain of the mare's reproductive status. Also, before the first ovulation, there is poor correlation between sexual behaviour and ovarian activity because it is common for the early heats to be unaccompanied by the presence of large follicles and some long spring heats are anovulatory. However, once ovulation has occurred, regular cycles usually follow with oestrus typically lasting 6 days of the 20 to 23 day equine cycle.

Cow

Puberty

Under conditions of domestication, normal and well-cared-for cattle are polyoestrous throughout the year. The age at first oestrus, or puberty, is affected by nutrition and season of birth and ranges from 7 to 18 months, with an average of 10 months. A small proportion of heifers do not ovulate at the first heat, and in a majority of young cattle, the oestrus associated with the first ovulation is 'silent' (Morrow et al. 1969). Poor feeding and calfhood disease delay puberty.

Seasonality

By definition, cows are polyoestrous and therefore not seasonal breeders. However, melatonin levels are influenced by photoperiod, and effects have been reported on milk yield, which is increased with long photoperiod; thus there may be more subtle effects on reproduction/reproductive performance (Dahl et al. 2000). Interestingly, once puberty has been reached, cyclical activity should persist, except during pregnancy, for 3 to 6 weeks after calving, during high milk yield (especially if there is some evidence of dietary insufficiency) and with a number of pathological conditions. Some cows and heifers also fail to show overt signs of oestrus yet have normal cyclical activity, a condition referred to as 'silent heat' or suboestrus. This may, however, be caused by failure of the herdsperson to observe the signs rather than a failure of the cow to show signs. The typically reported length of oestrus in heifers is 15 hours but with a wide range of 2 to 30 hours; the oestrous cycle is 20 days.

Sheep

Puberty

The onset of puberty in the ewe is typically around 8 to 9 months, but there is variation with breed and a strong influence of the time of birth as a result of photoperiod. For example, lambs born in the spring when natural food sources are good may reach puberty as early as 6 months. By contrast, those born later in the year may not reach puberty for 12 to 15 months.

Seasonality

The sexual season of most breeds of sheep in Britain is from October to February, during which time there are 8 to 10 recurrent oestrous cycles. The stimulus for the annual onset of sexual activity is declining length of daylight. The extent of the breeding season diminishes with increase in latitude; thus at the equator ewes may breed at any time of year, whereas in regions of high latitude – in both northern and southern hemispheres – the breeding season is restricted and distinct, with a prolonged phase of anoestrus after parturition. The breed of ewe also influences the duration of the breeding season; for example, in Dorset Horns it is distinctly longer than in other breeds – in which three crops of lambs can be obtained in 2 years – but in hill breeds, such as the Welsh Mountain and Scottish Blackface, it is shorter. Local breeds of central Europe and the Merino in Australia may not show an annual anoestrus. Also, in 'ordinary' breeds in Britain, such as the Clun Forest that normally shows a distinct seasonal activity, isolated instances of successful mating may occur in every month of the year. Ewe lambs and yearling ewes have shorter breeding seasons than older ewes. The seasonal onset of sexual activity can be advanced by artificial manipulation of the photoperiod and by the use of hormonal agents (described later in this chapter). Once cyclicity has been initiated, the interovulatory interval in British breeds is around 17 days and oestrus lasts about 30 hours.

Goat

Puberty

The onset of puberty in the doe goat varies from 4 to 8 months and is influenced by breed, the month of birth and the presence of a male. Similar to sheep, puberty usually occurs with reduced photoperiod in the autumn. However, does born in the spring will achieve puberty in autumn of the same year, whereas those born in autumn will not reach puberty until late the following year.

Seasonality

The breeding season in the UK is from August to February, with the greatest activity in the months of October, November and December. Near the equator, there is no evidence of seasonality but continuous cyclic activity. The doe (nanny) goat is polyoestrous, with an interoestrous interval of 20 to 21 days, although it is rather irregular at the beginning of the breeding season. The average length of oestrus is 36 hours, ranging from 24 to 28 hours.

Pig

Puberty

Puberty in gilts occurs at about 7 months, but diet, breed (including the degree of inbreeding) and season of birth influence the time of

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onset. At the first oestrus the number of ovulations is low, but it increases thereafter so that, if mating is delayed until the third heat, a larger litter will result. The crossbreeding of inbred lines increases the ovulation rate, as does the provision of a high energy diet for 11 to 14 days before the expected oestrus; however, continuing such a diet after mating increases embryonic loss. Fecundity is best from the fourth to seventh gestations.

Seasonality

Although the domestic sow is generally considered to be polyoestrous, the wild pig is a seasonal breeder, with the main period being late autumn and a second peak of activity in April (Claus and Weiler 1985). There is some evidence of the influence of photoperiod on reproduction in the domestic sow. For example, anoestrus is more prevalent in summer and to a lesser extent in February and March, whereas the ovulation rate is lower in summer. For the most part, the recurrent reproductive cycles of 21 days are interrupted by pregnancy and lactation. The average length of oestrus is 53 hours, with spontaneous ovulation occurring between 38 and 42 hours from the beginning (Signoret 1971).

Dog

Puberty

Puberty onset in the bitch ranges between 5 and 24 months and is influenced by breed, body weight and environmental factors. The smaller breeds of dog reach puberty earlier (5–6 months) than larger breeds, with little evidence of an effect of photoperiod on puberty onset in the domestic dog because it has lost the seasonality effects seen in wild canids (Gobello 2014).

Seasonality

Reproductive activity in the bitch differs from the polycyclic pattern of other species in that there are no frequent, recurring periods of oestrus, either repeatedly throughout the year as in cattle and pigs, or seasonally driven as in horses, sheep, goats and cats. In the domestic bitch, there is little evidence of a seasonal effect on reproductive function because there is a fairly even distribution of the occurrence of oestrus throughout the year (Sokolowski et al. 1977). In contrast, wild canids do exhibit seasonal influences on reproduction. For example, a study of captive African wild dogs has shown that there is evidence of seasonality, with the females exhibiting a biphasic pattern of receptivity manifest in the winter and summer (Newell-Fugate et al. 2012).

All bitches have a prolonged period of anoestrous or sexual quiescence between successive heats irrespective of whether they have been pregnant or not; this pattern has been described as monocyclic (Jochle and Andersen 1977). Despite extensive research, it is only comparatively recently that the mechanisms involved in determining the duration of anoestrus, and when bitches come into oestrus, have started to unfold. In addition, it must be stressed that this, as with much of the work on canine reproduction, has been done on experimental Beagle colonies, which may or may not be representative of the species in general. Recent evidence suggests that endogenous dopaminergic substances have a direct effect on regulating GnRH secretion, thereby stimulating basal FSH secretion and initiating folliculogenesis (Okkens and Kooistra 2006). The average interval between successive oestrous periods is 7 months, but it is variable. There is some evidence that the breed of the bitch can have an effect. For example, for the Rough Collie it is 37 weeks, but for the German Shepherd it is 26 weeks (Christie and Bell 1971); other breeds that were studied had mean intervals between these two figures. Mating does not appear to influence the interval, although pregnancy caused a slight increase (Christie and Bell 1971).

Cat

Puberty

Body weight is the most important factor in determining puberty in nonpedigree cats (Feldman and Nelson 1996). Females will usually show their first oestrus once a weight of 2.3 to 2.5 kg has been attained, at an approximate age of 7 months. Puberty occurs a month or two later in males, at a weight of approximately 3.5 kg. Puberty is also influenced by the season of birth; females born very early in the year may mature in the autumn of the same year, whereas those born later will not normally show oestrus until the following spring. Puberty is much more variable in pedigree cats (Jemmett and Evans 1977). Oriental queens (Siamese and Burmese) may show their first oestrus before 5 months of age, whereas pedigree long-hair cats may not mature until over 1 year of age.

Seasonality

Free-living nonpedigree and feral cats are seasonally polyoestrous, with a period of anoestrus that begins in the late autumn. Increasing daylight length is the most important factor in inducing the resumption of reproductive activity, and the first oestrus will usually be seen soon after the shortest day of the year. If a constant 14 hours of lighting is provided daily during the breeding season, sexual activity will continue throughout the year; this manipulation of photoperiod will alter the circadian rhythm of melatonin production (Leyva et al. 1984). If the lighting regimen is changed from 14 to 8 hours, then cyclic activity will cease immediately (Leyva et al. 1984). There may be a period of apparent lack of oestrous activity in the early summer, but this corresponds with the pregnancy or lactation after mating earlier in the year, rather than true anoestrus. In the cat, the duration of oestrus is 7 to 10 days and the interoestrous interval is about 17 days.

Artificial Induction of Puberty and Seasonality

In seasonal breeders, the ability to produce offspring out of season or to advance the time of onset of cyclic activity has advantages. In these and in other species, the ability to ensure that an individual or group of animals does not come into oestrus, or is in oestrus at the same time, has attractions. The methods that are available can be divided into two main groups: those that do not involve the use of hormones and those that do.

Nonhormonal Methods

Light

The onset of cyclical activity in the mare, ewe, goat and cat is dependent upon changes in the hours of daylight. The mare and queen are stimulated to activity by a lengthening photoperiod, whereas in the ewe and goat, it is the effect of a decreasing photoperiod that is the stimulus.

In ewes and doe goats, it is likely that it is the preceding long day length that controls the timing of the breeding season, with the decreasing day length actually synchronising the rhythm of the endocrine events that trigger the onset of cyclical ovarian activity. The provision of housing with controlled lighting enables the timing of the breeding season to be advanced or changed, even to the extent of providing an equatorial lighting regimen so that breeding

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can occur throughout the year. There are two approaches: (1) the gradual and (2) the abrupt. In the former, day length is reduced incrementally until the final fixed day length is reached. In the latter, the ewes and does are subject to a lighting regimen that on day 1 is the natural one, with a sudden decrease on day 2, and are then kept on reduced lighting until breeding has taken place. One method that obviates the need for special daylight-proof housing is if in late pregnancy or at parturition, ewes are exposed to 22 hours of daylight using an artificial source and then a reduction to natural day length. Alternatively, in late pregnancy ewes are exposed to 18 hours of daylight for 1 month, followed by a sudden return to 8 hours. This results in ewes having a fertile oestrus 3 months after lambing. Modifying photoperiod has not been used much commercially because it is not without problems, not least of which is the fact that there is a wide range in the response of the ewes as measured by the time of onset of cyclical ovarian activity. In the sow, an artificial reduction in day length from May to August has been shown to decrease the interval from weaning to oestrus from 23.6 to 5.7 days (Claus and Weiler 1985).

If mares are stabled in late autumn in the northern hemisphere and are subjected to artificial light, preferably of increasing duration, then it is possible to advance the onset of normal cyclical activity so that there is oestrus and ovulation. This effectively shortens the transition phase between anoestrus and the breeding season, a process that naturally takes place over several weeks or months. The recommendation for artificial lighting is to initiate it from 1 December at the latest. An economic, reliable and relatively easy way to manage artificial lighting is to expose mares to 2.5 to 3 hours of light at sunset. Day length itself does not appear to be the absolute factor in determining seasonality because mares naturally exposed to different periods of photostimulatory light have been shown to enter the breeding season at about the same time. Some studies not only suggest that 2.5 hours of additional light in the evening was sufficient to advance the breeding season, but also that additional lighting in the morning did not further advance the season (Sharp 2011). Of interest is that a 1-hour pulse of light after the onset of darkness appears only to be effective when applied 9.5 to 10.5 hours into the dark phase (Palmer et al. 1982). The mechanisms that underlie these effects are uncertain, although data is beginning to emerge on clock genes that appear to be regulated by the suprachiasmatic nucleus of the hypothalamus and coordinated throughout the body (Lowrey and Takahashi 2004). In a recent study, equine circadian clock genes (e.g., *PER1*: period homolog 1) were detected in hair follicles and shown to demonstrate 24 hour oscillations, thus providing a way of monitoring peripheral clock changes in the horse (Watts et al. 2012).

Nutrition

Because both photoperiod and nutrition influence reproduction at the level of the hypothalamus, altered nutrition is likely to have the capacity to influence reproductive parameters such as seasonality. For example, a study carried out over a 3-year period demonstrated that the well-fed mare has a longer period of cyclicity than those on a more restricted diet (Scaramuzzi and Martin 2008). Furthermore, the stabling of mares and the provision of good feeding are believed to assist in stimulating the onset of cyclic activity in early spring. There is also evidence for the converse because it has been reported that when yarded mares are turned out to fresh spring grass, about 80% of them come into oestrus and ovulate within 14 days (Allen 1978). Furthermore, barren and maiden mares maintained in yards on adequate, but mainly dried, feedstuffs during the winter and spring remain in anoestrus longer than those that are kept out at grass. An explanation for this is difficult to find, although it may be related to the beta-carotene content of the diet as fresh spring grass contains large amounts of this substance. Nutrition has also been reported to affect the seasonal pattern of activity in a number of other species, including goats and sheep, and can also influence the resumption of ovarian activity in postpartum cows (Scaramuzzi and Martin 2008).

Other Nonhormonal Methods

The presence of a male animal can exert its effect upon the cyclical activity of the female. This is well demonstrated in sheep, in which the introduction of a vasectomised tup at the start of the breeding season will stimulate the onset of oestrous cycles in the majority of ewes, as well as bringing about some degree of synchronisation of cyclical patterns.

Hormonal Methods

Kisspeptin

There has been considerable interest in kisspeptin as a stimulator of GnRH release. This has been investigated in a variety of species, including the rat, mouse, sheep, monkey and human. In the anoestrous ewe, a low dose infusion of kisspeptin stimulates gonadotrophin secretion and induces ovulation. Furthermore, kisspeptin is able to synchronise LH surges in progesterone-primed ewes (Caraty et al. 2007). This raises the possibility that kisspeptin or kisspeptin analogues may have a future use as pharmacological agents in the manipulation of the ovine reproductive axis.

In the anoestrous mare, equine kisspeptin-10 (eKp10) is able to induce LH and FSH secretion, but in contrast to the ewe, it is not able to induce ovulation. A similar response was observed when given to mares at different stages of the cycle (Decourt et al. 2014). Consequently, the role of kisspeptin in equine ovulation is less clear than in the sheep, which provides limitations on the kisspeptin system as a means of manipulating the equine reproductive axis. A novel kisspeptin-10 has also been identified in the dog, which is able to stimulate LH secretion when administered in vivo to the anoestrous bitch (Albers-Wolthers et al. 2014). Although dopamine–kisspeptin interaction has not been investigated in the bitch, this is a possibility because, in sheep, inhibitory dopaminergic neurons have been shown to block kisspeptin stimulation of GnRH during anoestrous (Goodman et al. 2012).

Although these experimental studies are promising in terms of future strategies to manipulate kisspeptin neurons to control puberty onset and seasonality, further research is required to fully understand the hypothalamic mechanisms involved.

GnRH

It seems sensible that repeated dosing with GnRH should induce oestrus in prepubertal females. However, there is little clinical justification for early induction of puberty, and efficacy is poor unless the female is close to puberty (i.e., is physiologically mature).

The reason for pubertal animals being acyclical may be a result of seasonality (e.g., mare, ewe, queen), monooestrous cyclicity (e.g., bitch) or postpartum anoestrus (e.g., cow).

For females in seasonal anoestrus, the repeated administration of GnRH or its analogues may induce a return to cyclical activity; however, efficacy is poor unless the female is close to a spontaneous cycle. For example, a study in the southern hemisphere (Williams et al. 2007) reported that low dose GnRH-releasing pumps advanced the onset of breeding activity in anovulatory mares. However, the greatest efficacy for oestrus induction in the mare is observed

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when treatment begins after the vernal transition (waves of follicle growth without ovulation) has already commenced. In clinical practice, exposure to artificial lighting regimes is required before implementation of GnRH regimes for termination of anoestrus.

For bitches in anoestrus, it is possible to induce oestrus using a GnRH superagonist (deslorelin) implant (Kutzler et al. 2009). The initial response to inserting a deslorelin implant is the release of FSH and LH, which causes follicle growth and results in a return to oestrus. At this point, the implant needs to be removed to prevent later receptor down-regulation (the deslorelin implant is marketed as a contraceptive). Fertility with deslorelin-induced cycles in bitches appears good.

Anoestrus, common in postpartum animals, can be a particular concern in the cow, in which the principle causes are negative energy balance and stress, which adversely affect systemic concentration of metabolic hormones, insulin and insulin-like growth factor. In these cases there is follicle growth but a lack of ovulation. Single doses of GnRH have limited success at terminating postpartum anoestrus in dairy cows; rather, regimes using GnRH to cause luteinisation of the anovulatory follicles are followed by prostaglandin administration to cause luteolysis of the luteinised follicle (see Chapter 25).

Gonadotrophins

For induction of oestrous cyclicity in pubertal animals, the repeated administration of crude pituitary extracts or of equine chorionic gonadotrophin (which is mainly FSH-like in activity) has moderate success, notwithstanding the underlying causes of anoestrus noted previously (seasonal breeders, nonoestrous breeders, postpartum nutrition and stress). In these circumstances, modification of the underlying situation is the most important aspect of clinical management. Furthermore, notice should be paid to the important role of progesterone in the establishment of normal cyclicity in many species (see species overviews earlier in the chapter). For example, the use of eCG alone to induce oestrus in seasonally anoestrous ewes is not very successful. However, if progesterone is administered to the ewes before the injection of eCG, then there is synchronised oestrus and ovulation. Perhaps surprisingly, eCG does not appear to stimulate ovarian activity when given to mares in winter anoestrus. The reasons for this are probably twofold. First, it may be that the dose required to stimulate follicular development is large, and second, eCG has both FSH and LH activity in the mare and so may cause follicle luteinisation rather than ovulation.

Melatonin

The pineal gland controls reproductive activity in seasonal breeding species such as sheep, goats, horses and cats by the secretion of melatonin. The administration of melatonin cannot be used to induce seasonal activity in the long-day breeders because it would be necessary to inhibit the secretion of melatonin or neutralise its effect (in fact in the queen melatonin implants can be used to suppress oestrous cyclicity). Termination of melatonin secretion can be most easily affected by exposure to appropriate artificial lighting regimes (see earlier mention). However, in the ewe and doe, which are short-day breeders, melatonin has been used commercially to advance the timing of the onset of the breeding season. The hormone is administered as an implant containing 18 mg of melatonin that is inserted subcutaneously at the base of the ear. It is critical that rams (and bucks) should be separated from the ewes so that they are out of sight, sound and smell at least 7 days before the insertion of the implant. They must remain separated for at least 30 days, but not more than 40 days, when rams (or bucks) should then be reintroduced. Peak mating activity occurs 25 to 35 days later. The breeding season can be successfully advanced by 2 to 3 months with good fertility. Melatonin should not be used in ewe lambs because its use can significantly delay the onset of puberty.

Progesterone

The physiological role of endogenous progesterone in initiating normal ovulatory cycles has been described previously. Exogenous progesterone may be used in anoestrous females to induce oestrus, as long as the species is polyoestrous. Consideration should always be given to the underlying causes of the anoestrus as described previously. Although detailed regimes are described in the species-specific chapters of this book, the broad principles are that progesterone is given continually over a period of time similar to the normal luteal phase, thus mimicking the luteal phase such that, when progesterone is withdrawn, the events of a normal follicular phase will be stimulated. Progesterone or progestogens are marketed as oral preparations given daily (e.g., for mares and sows), vaginal sponges (e.g., for ewes) or as vaginal devices (e.g., for cows). Progesterone alone is not highly efficacious in treating anoestrus because some individual animals: (1) have insufficient follicular activity at the beginning of treatment; (2) ovulate spontaneously during treatment; and (3) have follicle growth but fail to ovulate after withdrawal of the progesterone. A variety of species-specific regimes have therefore been developed to overcome these problems. These include: (1) before progesterone treatment starts, ensuring follicle activity by adequate lighting (e.g., mare) or administration of GnRH preparations (e.g., cow); (2) causing lysis of any spontaneous luteal tissue by administration of prostaglandin at the time of progesterone withdrawal (e.g., cow); and (3) ensuring that induced follicle growth results in ovulation by administration of GnRH or hCG shortly after progesterone withdrawal (e.g., mare, cow, ewe).

Oestrogens

Administration of exogenous oestrogens can cause signs of behavioural oestrus in many species. In cows, oestrogens have been used in combination with progesterone in the belief that they augment the signs of oestrus when progesterone is withdrawn (see previous mention); however, they have no role in inducing follicular activity.

A number of anecdotal reports suggested that low dose oestrogen could induce oestrus in anoestrous bitches. This was confirmed by Bouchard et al. (1993), who found that diethylstilboestrol administered orally at 5 mg/day/dog induced oestrus within 6 to 9 days and that pregnancy rates and litter size were normal (Bouchard et al. 1993). This work has not been progressed further because of the risk of adverse effects of oestrogen and the relative safety of using prolactin inhibitors (see next section).

Prolactin Inhibitors

In bitches, prolactin is the principle luteotrophic factor during the luteal phase. Daily administration of prolactin inhibitors, such as cabergoline and bromocriptine during the luteal phase, causes a rapid decline in progesterone concentrations, and the bitch enters anoestrus. Surprisingly, if administration continues during anoestrus, there is a significant shortening of anoestrus, and the bitch returns to oestrus. If treatment begins in the luteal phase, administration may be required for 8 to 10 weeks, and pregnancy rates are approximately 60%. However, when treatment begins in anoestrus, administration may only be required for 6 weeks, and pregnancy rates are greater than 80% (Spattini et al. 2007). The exact mode of action is unknown, but prolactin inhibitors appear to be the most useful method of oestrus induction for clinical practice.

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4

Fertilisation and Development of the Conceptus

MARCEL TAVERNE

Development of the Conceptus

Within the Uterine Tube

As the ripe follicle is about to rupture, the fimbriated end of the uterine tube partially envelopes the ovary at ovulation when the follicular fluid and egg are discharged. In many domestic species those sperm that traverse the female tubular genital tract are sequestered in the isthmus of the uterine tubes and are only released and migrate to the ampullae of the tubes (where fertilisation occurs) at the start of ovulation. Thus sperm's ascent and oocyte's descent to the ampulla are synchronised. Fertilisation requires that there is fusion of nuclear material from only one spermatozoon and one oocyte, although many thousands of spermatozoa will be released from sequestration in the isthmus at the same time; thus there will be many present in the ampullae at the time of fertilisation. The oocyte has mechanisms to prevent fertilisation by more than one spermatozoon (polyspermy) because if this occurs, it will result in the abnormal development of the embryo (see Chapter 9). This mechanism is called the 'polyspermic block'.

After fertilisation, cleavage of the zygote begins, and as a result of peristaltic contractions and ciliary currents in the uterine tube, it is propelled toward the uterus. When it reaches the uterus, at 3 to 4 days in cattle and at 5 to 8 days in the dog and cat, the zygote consists of 16 to 32 cells in the form of a morula. With further cell division and cell orientation, the morula becomes 'hollowed out' to form a blastocyst, and its wall becomes two layered; the inner is the endoderm and the outer is the ectoderm, and the cavity is known as the blastocoele (Fig. 4.1 and 4.2A). The embryo will form from a thicker portion of the ectoderm, referred to as the embryonic knot or disc (see Fig. 4.1 and 4.2A and B). The remaining part of the ectoderm is referred to as the trophoblast (from the Greek meaning nourishing layer) and will have an important subsequent role in the formation of the placenta (Perry 1981).

Up to the time of shedding of the zona pellucida at the ninth day (often called 'hatching') (see Fig. 4.1), there is little absolute growth of the mammalian egg from its original dimension of 0.14 mm. The fertilised eggs of the ewe enter the uterus at the eight-cell stage on day 3, whereas in the sow they progress down the uterine tube within 2 days of ovulation, arriving in the uterus at the four- to eight-cell stage (see Fig. 4.1). Tubal transport of the fertilised eggs of the mare probably takes 5 to 6 days, by which time they are at the blastocyst stage; van Niekerk and Gernaeke (1966) showed that unfertilised equine eggs remain in the tubes for several months, where they slowly degenerate. The variable duration of travel by the fertilised egg in the uterine tube of the domestic species appears to be determined positively by the degrees of activity of the tubal muscle and cilia and negatively by the muscular constriction, either at the tubal isthmus or at the uterotubal junction. Both positive and negative factors are probably influenced by variable concentration of ovarian steroid hormones and possibly by locally produced prostaglandins.

Within the Uterus

From the time of its arrival in the uterus until attachment, the zygote is propelled or aspirated into the uterine lumen, where it is nourished by the exocrine secretions of the uterine glands referred to as 'uterine milk'. In the polytocous species the blastocysts are distributed throughout the uterus so as to utilise the uterine space effectively; thus there occurs free migration of embryos between the cornua, regardless of the side of ovulation. In the monotocous cow such migration rarely occurs, and in the ewe it is not uncommon, whereas in the mare extensive migration always occurs (see later in this chapter). After the ninth day, the blastocyst elongates rapidly in ruminants and pigs. For example, the sheep blastocyst is 1 cm long at 12 days, 3 cm at 13 days, and 10 cm at 14 days, whereas in the sow at 10 days just after hatching it is a flaccid sphere 8 to 10 mm in diameter, by day 13 the apparent length of each blastocyst is 20 to 30 cm, but because of the folding of the mucosal surface, it may be a metre or more in length; the diameter is very small in comparison. A characteristic feature of the elongated porcine blastocyst is that it is always aligned with the uterine attachment of the broad ligament. By day 21 in the mare, the blastocyst measures 7 x 6.5 cm, but in the cow it extends almost throughout the entire pregnant horn. Embryonic attachment to the uterus occurs at approximately these times: 12 days, cow; 18 days, sow; 15 days, ewe; 13 to 17 days, dog and cat; and 35 to 40 days, mare.

A tough, elastic glycoprotein capsule surrounds the equine blastocyst between 6 and 23 days after ovulation, which provides physical support for the embryo as it traverses the uterus; it also accumulates the constituents of the 'uterine milk' (Allen & Stewart 2001). The unique intrauterine mobility of the equine embryo has been demonstrated by means of B- mode real time transrectal ultrasonography by Leith and Ginther (1984). The location of the vesicle was observed in seven mares over a 2-hour period daily from days 9 to 17. There was increasing mobility from day 9 to day 12, and a plateau of high mobility persisted until day 14,

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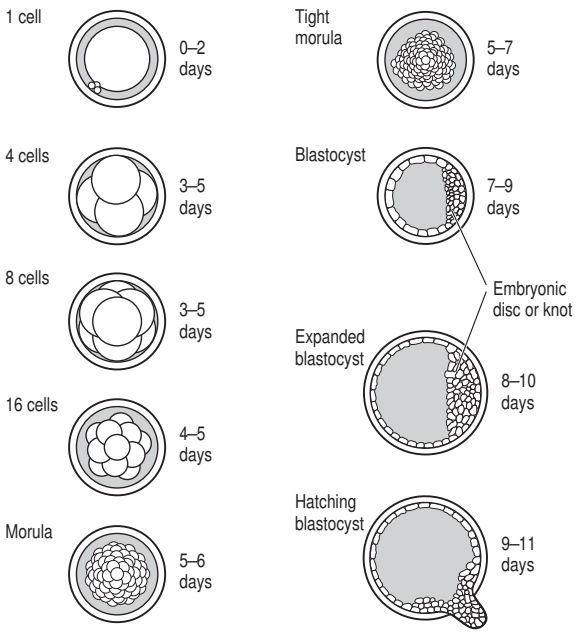
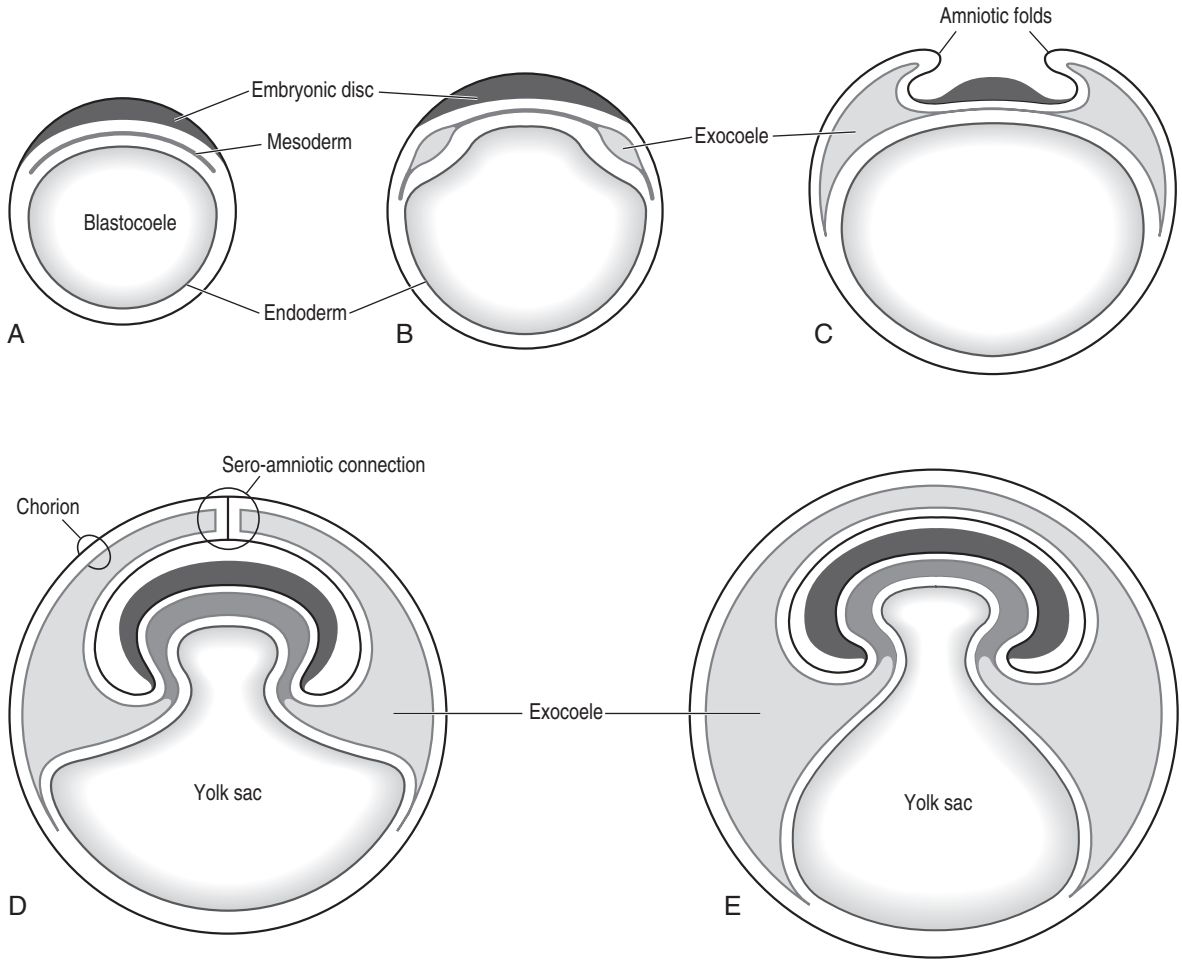


• **Fig. 4.1** Stages of early embryonic development in domestic species and their approximate timings.

after which there were few location changes on day 15 and none on days 16 and 17, indicating that fixation has occurred at the base of one of the uterine horns and is due to increased uterine tonicity. On days 9 and 10 the vesicle was at least twice as likely to be in the uterine body as in a uterine horn; on days 11 to 14 this proportion was reversed, and beyond day 15 the embryo was always cornually disposed.

Formation of the Fetal Membranes

A detailed knowledge of the formation and structure of the fetal membranes is crucial for a correct interpretation by manual palpation and for visual interpretation of ultrasound images of early pregnancy (see Chapter 5). In addition, early embryonic structures are involved in the dialogue between the embryo and the uterus, thus enabling the maternal recognition of pregnancy (see Chapter 5), implantation, placentation, and growth of the conceptus. At around the time of the blastocyst 'hatching' the embryonic (subsequently fetal) membranes develop, some of which will have a major role in the formation of the placenta. These are: yolk sac, amnion, chorion, and allantois. Soon after 'hatching', the inner (endodermal) layer of cells of the bilaminar blastocyst (see Fig. 4.2C), usually formed by the tangential division of a few cells of the embryonic disc, migrate around the inner surface of the ectoderm



• **Fig. 4.2** Development of the amnion and chorion, and the extension of the exocoele around the yolk sac shown in transverse section in the pig. In (D) the amniotic folds have met, but not yet fused, so that the amnion and chorion are joined by a 'sero-amniotic' connection. (From Perry 1981.)

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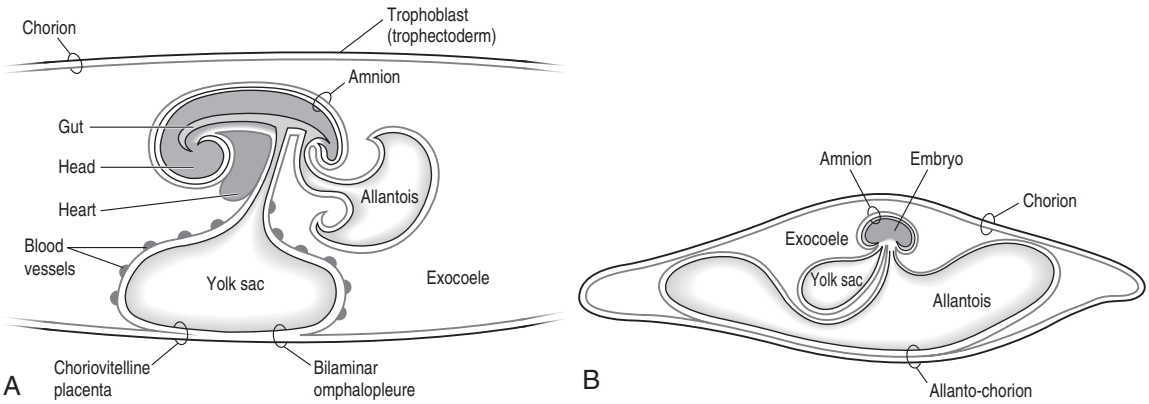

• **Fig. 4.3** (A) Fetal membranes of the pig as shown in longitudinal section on day 18 after mating and (B) on day 21 after mating.

and the blastocoele to form the yolk sac (see Fig. 4.2D). In eutherian mammals, and thus in all of the common domesticated species, it contains no obvious yolk. In mares the yolk sac will have been formed completely by day 11, and its spherical dimensions rapidly increase until day 20. The yolk sac fluid of the equine embryo is the first recognisable structure that can be identified during early ultrasonographic pregnancy diagnosis (see Chapter 5). The equine yolk sac fluid is hypertonic and contains high levels of fructose, proteins, and prostaglandins. The wall of the yolk sac produces IGF-1 and synthesises and metabolises steroids and prostaglandins (Betteridge 2007, Stout & Allen 2002). In the pig the yolk sac reaches its maximum size at about 18 days, but by 20 days, probably associated with its separation from the trophoblast, it has shrunk (Fig.4.2D and 4.3A and B; Perry 1981). In the mare, dog, and cat the yolk sac persists for a much longer time and can be easily recognised during ultrasonic scanning. The amnion develops as an enveloping fold of ectoderm and mesoderm, ultimately fusing and completely surrounding the embryonic disc, embryo, and fetus (see Fig. 4.2E and 4.3A and B); it is complete, for example, in the pig by day 17 and in mares it can be recognised during scanning from about day 30 onwards. Although the amnion has some involvement in the intramembranous absorption of amniotic fluid and solute into the fetal circulation (Brace et al. 2004), its main role is protective, containing the amniotic fluid in which the fetus 'floats' and/or moves.

At around the same time the chorion is formed by the outer layer of mesoderm and trophoblast (see Fig. 4.2D). The last of the embryonic/fetal membranes to form is the allantois, which develops as a thin walled, sac-like outgrowth of endoderm surrounded by mesoderm, from the embryonic gut close to the opening connecting the gut with the yolk sac; in the pig it starts to develop at about 14 days (see Fig. 4.3A and B). The allantois grows very rapidly, passing through the umbilical cord and appearing as a crescent-shaped sac filling the exocoele, except for the region around the embryo where the amnion is in contact with the chorion (see Fig. 4.3A and B). The mesoderm covering the allantois fuses with the mesoderm lining the chorion to form the allantochorion (chorioallantois), the structure which is involved in the formation of the placenta (Figs 4.3B, see also 4.5A; Perry 1981). The allantois also fuses with the amnion, to form the allantoamnion. In mares the early development of the allantois can be visualised with transrectal ultrasonic scanning; around day 29 the allantois occupies about 50% of the oval-shaped conceptus, with the embryo in the middle of a tissue line consisting of the apposed walls of allantoic and yolk sacs (see Fig. 5.2D; Ginther 1995, Allen & Wilsher 2009). Detailed overviews of the development and final state of the yolk sac, amnion, allantois, and chorion in different mammals have recently been published (Carter & Enders 2016, Chavatte-Palmer & Tarrade 2016).

Ruminants

After hatching of the blastocyst the bovine embryo, although differentiating fast, elongates slowly compared with the chorion, and at 1 month after mating it is only just over 1 cm long. The chorionic vesicle, which is at first string-like with a central distended sphere of amnion containing the embryo, is progressively filled by allantoic fluid to form an extensive allantochorionic sac; this already occupies the nonpregnant horn at the end of the fourth week of gestation. Recognition of this allantoic fluid enables early ultrasonographic pregnancy diagnosis between days 20 and 25 to be made (see Chapter 5). Because of its expansion, the allantoic sac begins to distend the gravid uterine horn from about day 35 onwards (Fig. 4.4A). At this stage, it can also be palpated manually, together with the amniotic vesicle. At this time the chorion already extends into the nongravid horn; its length is about 40 cm, and at its widest part in the dependent portion of the gravid horn, it is 4 to 5 cm in diameter.

The early development in sheep is very similar to that of the cow. The allantochorion, which eventually surrounds the allanto–amnion, is separated from it by allantoic fluid. When the vascularisation of the chorion by the allantois is complete (at 40–60 days in the cow), the allantochorion is ready to participate in placental function (see Figs.4.4A–C, and 4.5). Before this time the embryo has been nourished through its chorion and amnion by diffusion from the uterine milk. For a detailed description of the early development of the bovine fetal membranes and placenta see Assis-Neto et al. (2010) and Peter (2013).

In the ruminant uterus, where the allantochorion contacts the uterine caruncles, finger-like processes or villi containing capillary tufts grow out from the allantochorion into the crypts of the maternal caruncles, which are also surrounded by capillary plexuses. Thus is formed the characteristic ruminant placentome, through which nutrient and gaseous exchange between the mother and fetus takes place (Fig. 4.6). There can be up to 120 functioning cotyledons in the cow (Figs 4.4, 4.5, 4.7, and 4.8) and about 80 in the sheep, which are arranged in four rows along each of the uterine horns. As already mentioned previously, the chorion and, preceding it, the allantois, both extend into the nongravid horn,

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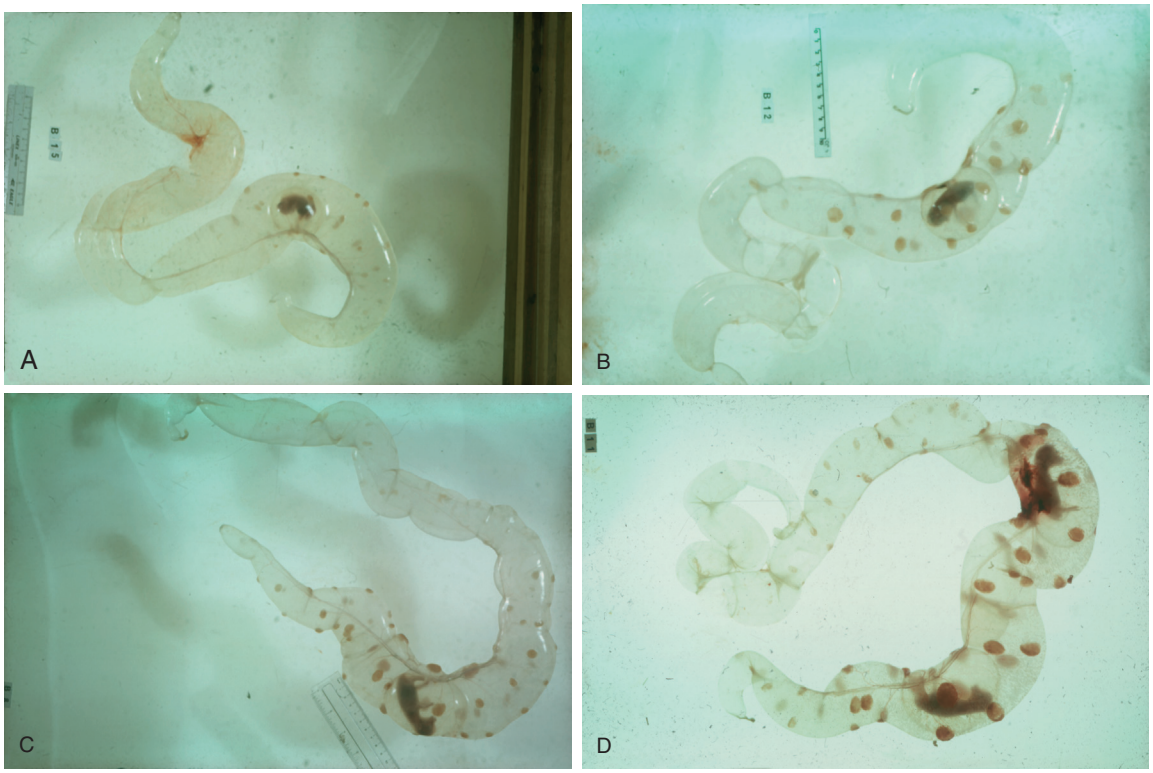
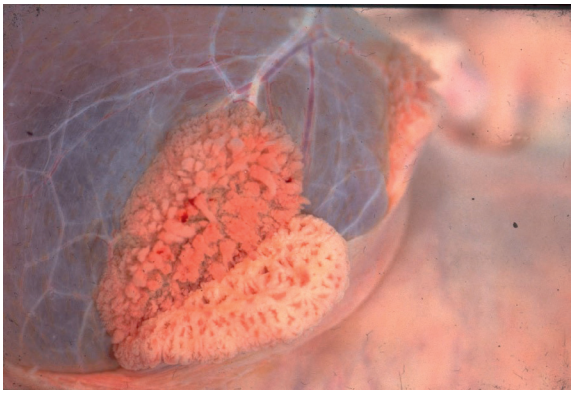


• **Fig. 4.4** Bovine conceptus at: (A) 40 to 45 days; (B) 50 days, CR length 5.5 cm; (C) 80 days, CR length 8 cm; (D) Twin conceptuses at 60 to 70 days, CR length 7.0 and 7.6 cm. Note the fetus contained within the discrete – almost spherical – amniotic sac, with an elongated allantochorion, and evidence of the formation of cotyledons.

• **Fig. 4.5** Bovine conceptus at 90 to 100 days. Note the relatively larger amniotic sac compared with Fig. 4.4 A, B and C, and obvious cotyledons which are larger towards the centre of the allantochorion.



• **Fig. 4.6** A cotyledon, consisting of finger-like processes (villi) of the vascularised allantochorion *(blue in colour)*, is manually separated from crypts in a caruncle of the uterine wall *(lower part of the picture)*.

and thus it is normal in the ruminant for there to be numerous functioning placentomes in the nonpregnant horn.

During early development of the ruminant embryo, there occurs an extensive fusion between the allanto–amnion and the allantochorion (forming the so-called 'amnio-chorion'), thus largely obliterating the cavity of the allantois. As a result of where it lies over the amnion, the allantois is reduced to a narrow channel. Here its shape resembles the letter 'T', with the stem coming out of the urachus along the umbilical cord and then diverging as the two crosspieces over the lateral face of the amnion. Consequently, there is little allantoic fluid over the amniotic area; most of it lies in the extremities of the allantois, one of which lies in the nongravid horn (see Figs 4.7 and 4.8). However, studies of bovine uteri in late gestation (Arthur 1959) have shown that, with the increasing pressure of accumulating allantoic fluid, the allantochorion tends to become separated again from the allanto–amnion, so that at term, the allantois may almost surround the amnion.

Horse

The equine conceptus does not show the initial rapid elongation of the blastocyst–chorion, as occurs in ruminants and pigs. For example, at 35 days the equine chorion is oval rather than cylindrical and is more distended by the allantoic fluid. This causes an earlier, more discrete uterine enlargement than in the cow, and

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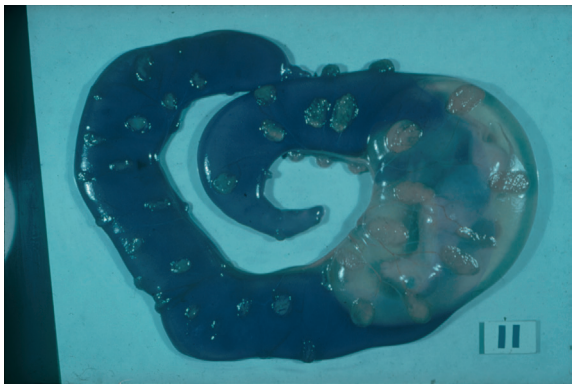
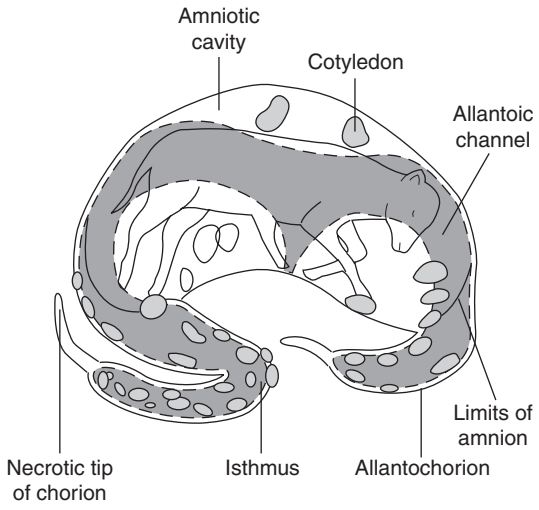
this is helpful in early clinical pregnancy diagnosis (Fig. 4.9). In the mare, unlike the cow, the amniotic and allantoic sacs do not fuse as in ruminants, so that the amnion floats freely, contained within the allantoic sac except for its attachment at the umbilicus, throughout gestation (Figs 4.10A and B). The relationships of the fetal sacs at birth will be referred to again in Chapter 6 on parturition.



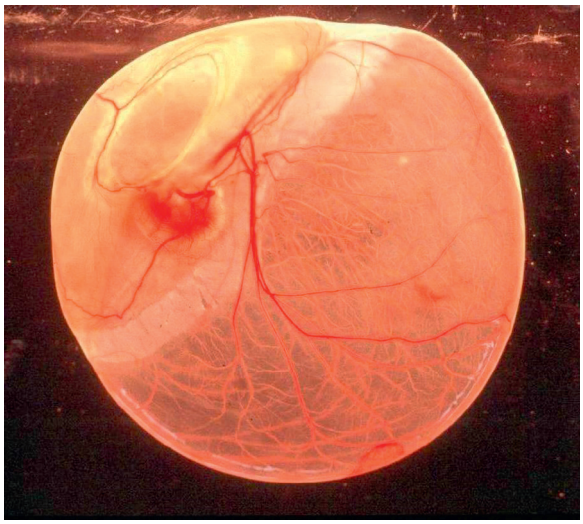
• **Fig. 4.7** Bovine conceptus at about 100 days, in which methylene blue dye has been injected in to the allantochorionic sac post mortem.



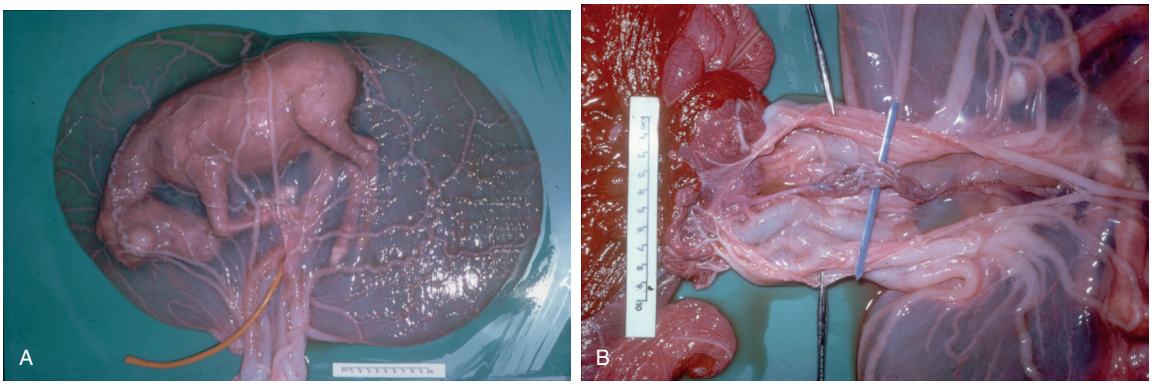
• **Fig. 4.8** Bovine conceptus showing the allantochorion of early pregnancy. (From Zeitzschmann 1924.)

In the mare, after the establishment of a stable microvillus contact between the allantochorion and endometrium at about day 40, there develops over the next 100 days a complex of multibranched interdigitations with the endometrium to form what are referred to as microcotyledons (Samuel et al. 1975, Steven 1975). Although more or less fully differentiated by day 150, there is continued lengthening and branching during the second half of gestation (McDonald et al. 2000). The microcotyledons are distributed over the whole surface of the allantochorion and can be seen with the naked eye, giving the allantochorion a stippled, velvety appearance (Figs 4.11A–C and 4.12). Between the microcotyledons are areas of pseudostratified trophoblast cells that overlie the ducts of the endometrial glands, absorbing their secretions (Steven 1982).

At day 35 the trophoblast of the spherical conceptus has separated into its invasive and noninvasive components. Cells from the former, distinguished as the thickened annulated chorionic girdle (see Fig. 4.9), invade the endometrium to form the endometrial cups, structures unique to equines (Allen & Stewart 2001). These appear as crateriform structures, which are disposed in a concentric manner at the base of the pregnant horn. They are present from



• **Fig. 4.9** Equine conceptus at 5 weeks. Between the vascularised allantochorion (on the right) and yolk sac (upper left), the embryo can be recognised. The white band between the two sacs is the chorionic girdle from which cells will migrate into the endometrium to form the endometrial cups. (Courtesy of Professor W.R. Allen.)



• **Fig. 4.10** (A) Equine fetus of approximately 3 months of age. (B) The allantochorion has been incised to expose the fetus enclosed within the almost transparent amnion. Note the umbilical cord at the bottom of the picture and the tortuous blood vessels on the surface of the amnion.

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• **Fig. 4.11** (A) Full-term equine fetus within the intact chorionic membrane. The hindlegs are contained within the part of the chorionic sac that occupied the pregnant horn. The arrow indicates the part of the allantochorion that occupied the contralateral horn. (B) The allantochorionic sac has been incised and reflected *(arrow on the right)* to expose the fetus enclosed within the intact *vascularised* allantoamnion; note the part of the umbilical cord that is covered by the allantois *(arrow in the middle)*. (C) The allantoamnion has been opened so that the part of the umbilical cord that is covered by the allantoamnion can be seen *(arrow)*.

• **Fig. 4.12** Outer surface of the allantochorion, showing a white star which was adjacent to the internal opening of the cervix and is devoid of placental villi. This image is of the part of the chorion overlying the head of the near-term fetus of Fig. 4.11A.

the 6th to the 20th week of gestation, and in them the equine chorionic gonadotrophin (eCG) is produced. The cups express foreign fetal antigens (they are fetal trophoblast cells), stimulating strong maternal humoral and cell-mediated immune responses, which ultimately curtail their lifespan; they regress and disappear at about day 140, although the timing of this varies (Allen & Wilsher 2009).

The immunological importance of the endometrial cups in protecting the 'foreign' conceptus has been demonstrated by Allen (1982). In interspecies transfers of fertilised eggs between horses and donkeys, no endometrial cups were formed, and the donkey fetuses died at 80 to 90 days. A final stage of the regression of the endometrial cups can also be recognised by the formation of so-called allantochorionic pouches (Ginther 1992). Projecting into the allantoic fluid, these are peculiar invaginations of the allantochorion, which are first found at a fetal body length (FBL) of about 11 cm, and occur in juxtaposition with the endometrial cups. Their size corresponds with the secretory activity of the endometrial cups, being largest at FBL 15 to 20 cm, and regressing after FBL 30 cm. They are few in number and are not pathological structures.

The surface of the allantochorion, adherent to the endometrium, is red in colour with a 'velvety' appearance and texture. The area adjacent to the internal opening of the cervix is devoid of placental villi, giving rise to the so-called called 'star' (see Fig. 4.12). The inner surface of the allantochorion, which is outermost when the placenta is shed, has a smooth surface (see Fig. 4.11). Sometimes attached to the allantochorion or floating freely in the allantoic fluid, there are soft calculi of cellular and inorganic debris called

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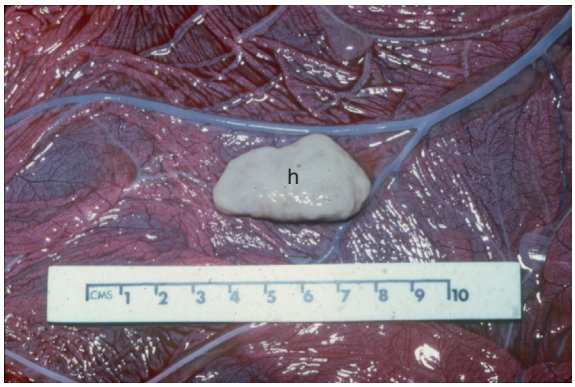
hippomanes (Fig. 4.13); they consist of concentric layers of cellular debris and appear to have no physiological function (Whitwell & Jeffcott 1975).

Pig

The development of the fetal membranes has been extensively studied in the pig and very clearly described by Perry (1981) as well as in a textbook on comparative placentation (Steven 1975). After the blastocysts have hatched at day 7, they enlarge so that on day 10 they are flaccid spheres, 8 to 10 mm in diameter. These are then distributed and spaced along both horns (Dziuk 1985) before becoming 'fixed' on day 12 by 'adhering' to the endometrium near the attachment of the broad ligament. Over the next 1 to 2 days, there is a huge transformation of the conceptus in that it undergoes rapid elongation, forming a very long narrow cylinder. Although occupying 20 to 30 cm of horn length, because of the extensive folding of the endometrium, the blastocyst may actually be over a meter in length. The amnion forms from 10 days, and the allantois first appears at about 14 days, expanding into a voluminous crescentic vesicle by 18 days; by 19 days it has fused with the chorion to form the allantochorion. By 22 days the allantochorion has developed intricate corrugations, which interlock with the folds and crevices of the endometrium to establish the placenta (Marrable 1969, Dantzer & Leiser 1994). There is a rapid accumulation of fluid within the allantoic cavity between day 20 and 30 (Figs 4.14A–C and see Fig. 4.18). The presence of this fluid in the allantochorionic sacs of the multiple fetuses allows the early pregnancy diagnosis with ultrasonic scanning (see Chapter 5).

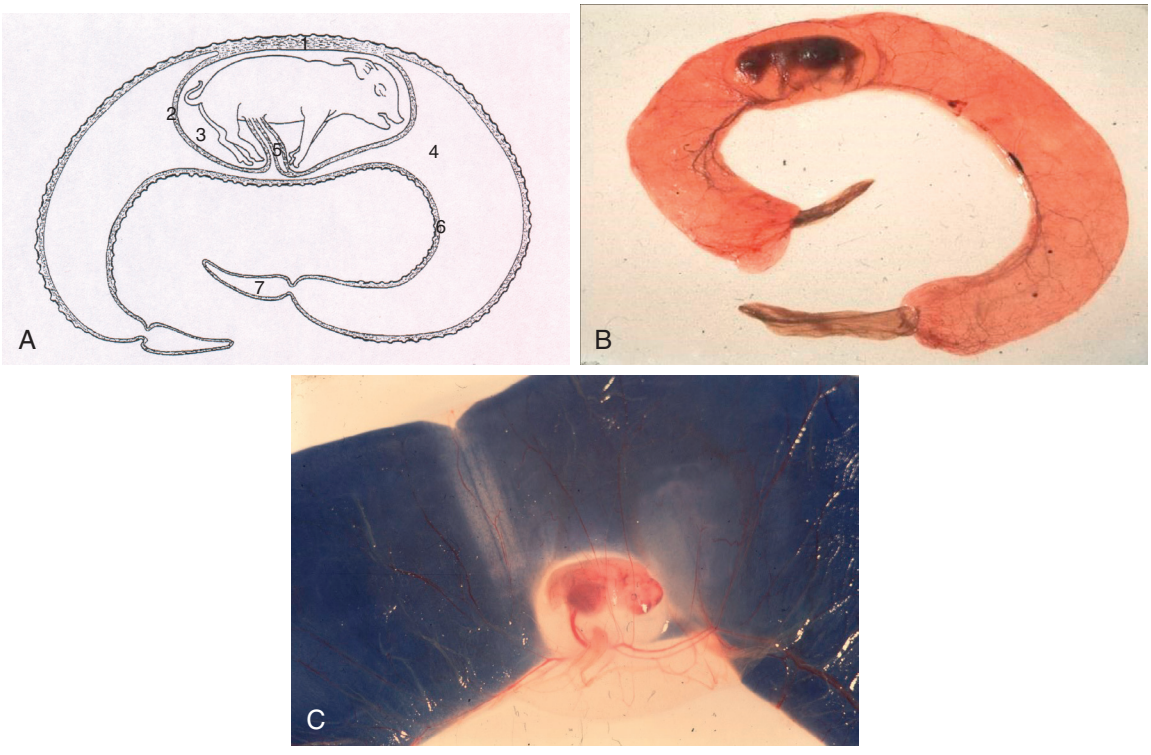


• **Fig. 4.13** Allantochorionic membrane of the mare with hippomane *(h)* attached.

Dog and Cat

After intrauterine and transcornual migration between days 12 and 17 after the LH surge, fixation and implantation begins by days 17 to 19 in dogs (Pretzer 2008).

Until 20 days of gestation, there is a choriovitelline placenta in which the vascular yolk sac is involved. Even at 24 days, the yolk sac is 3 times the length of the embryo and can easily be recognised during ultrasonic scanning (see Chapter 5). The embryo is completely surrounded by the avascular amnion, which contains translucent fluid, and is in contact with the yolk sac via the vitelline duct. From 20 days the allantois develops, and by day 22 it is twice the size of the yolk sac and contains a clear yellow fluid; it completely envelopes the amnion and thus the embryo and fetus. The central part of the allantoic sac, having fused with the



• **Fig. 4.14** (A) Fetal membranes of the pig, shown in longitudinal section: *(1)* amniochorion; *(2)* allantoamnion; *(3)* amniotic cavity; *(4)* allantoic cavity; *(5)* urachus within the umbilical cord; *(6)* allantochorion; *(7)* necrotic nonvascularised end of the chorion. (B) Complete conceptus at about midpregnancy. (C) A blue stain has been injected into the allantoic cavity at about day 30; the outline of the amniotic cavity and the blood vessels in the umbilical cord are clearly visible.

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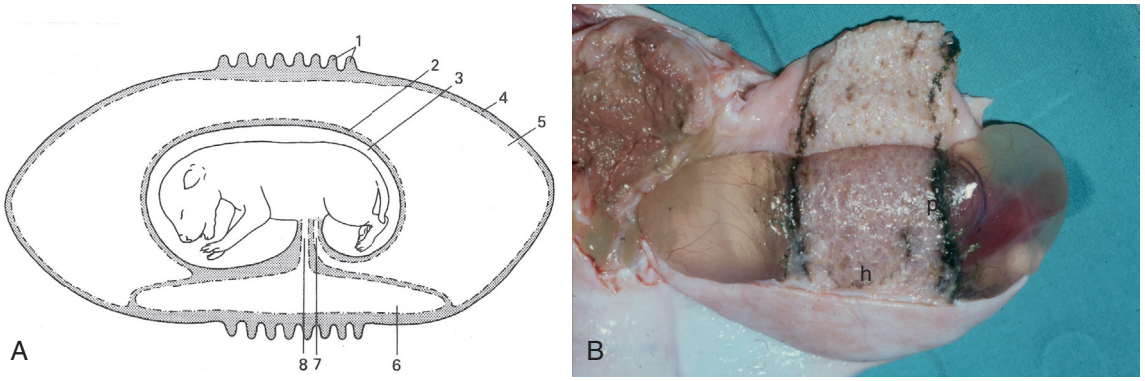

• **Fig. 4.15** Fetal membranes of the dog, as shown in longitudinal section, during the 7th week of gestation (A): *(1)* allantochorion involved in the formation of the zonary placenta; *(2)* allantoamnion; *(3)* amniotic cavity; *(4)* allantochorion outside the zonary placenta; *(5)* allantoic cavity; *(6)* yolk sac; *(7)* urachus, connecting the allantoic cavity with the bladder of the fetus; *(8)* stalk of the yolk sac within the umbilical cord. (B) Canine conceptus at approximately 8 weeks of gestation, partially contained within part of the uterine horn, showing marginal haematomata on the allantochorionic sac *(h)* and the areas of placentation *(p)*.

chorion and which is in contact with the endometrium, forms the epithelial chorial zonary placenta. In the dog it completely encircles the embryo (Fig. 4.15A), but in the cat it is incomplete.

Types of Placentation

Placentae may be classified according to the way the villi are distributed on the fetal chorion. Thus when they are uniformly dispersed, as in the mare and sow, the placenta is said to be diffuse. When they are grouped into multiple circumscribed areas, as in the ruminant, the placental arrangement is called cotyledonary, whereas in the bitch and cat the villi are disposed in the form of a broad encircling belt forming a zonary placenta. Formerly, the placentae were differentiated according to whether or not maternal tissue separated from the fetal tissue at birth. Thus of the domestic mammals the placentae of the bitch and cat were said to be deciduae and those of the remainder nondeciduae. More commonly, embryologists favour Grosser's (1909) division of placental types, in which the degree of proximity of the maternal and fetal blood circulations is the criterion of classification. Such a concept recognises the phagocytic property of the trophoblast, or chorionic epithelium that may be exerted on tissues with which it comes into contact. In the simplest, or epitheliochorial type of placenta, seen in the horse and pig, the chorion is everywhere in contact with the endometrium, and there is no loss of maternal tissue during the formation of the placenta. In the cow the placenta is described as being synepithelialchorial (Wooding 1992, Leiser & Kaufmann 1994). Soon after embryonic attachment a syncytium is formed on the maternal side of the placentomes by the fusion of binucleate cells, derived from the trophectoderm, with uterine epithelial cells. Unlike the sheep and goat, the syncytium in bovine placentomes is only temporary, as fairly soon the syncytial plaques are overgrown by the rapid division of the remaining maternal epithelium (King et al. 1979). In the third endotheliochorial type, there is further invasion of the endometrium by the trophoblast, which is now apposed to the maternal capillaries. Such a type is typical of the carnivora. In the haemochorial placenta, only the tissues of the chorionic villi separate the fetal and maternal blood. According to the number of trophoblast layers, these structures were further subdivided (Enders 1965) into haemotrichorial (rat, mouse, and hamster), haemodichorial (lagomorphs and early human placenta), and haemomonochorial (cavia, higher apes, and human placenta at term). The placenta of the dog and cat is partly haemochorial in that the main zonary placenta, which is of endotheliochorial type, is flanked by marginal haematomata. At about 22 to 25 days, they are visible at the margins of the of the placental girdle. In the dog they are green in colour due to the presence of the blood pigment uteroverdin and are 1 mm width at this stage but reach 8 mm at term (see Fig. 4.15B). In the cat they are less obvious, although they can be seen microscopically; the pigment is brown in colour. The role of these haematomata is believed to be the transfer of iron to the fetus. In the majority of cat placentas, there is a well developed fissure similar to that seen in mink; its function is not known (Miglino et al. 2006). When separation of the canine placenta begins at parturition, it is the escape of this altered blood from the marginal haematoma that gives the characteristic green colour to the normal parturient discharges.

A simple diagrammatic representation of the types of placenta, based on Grosser's classification, is shown in Fig. 4.16. The degree of intimacy of the maternal and fetal placental blood vessels is the basis for the variable function of the 'placental barrier' of different species. This is of interest in certain diseases of the fetus and newborn. For example, in the haemolytic disease of foals, antigens from the fetus pass across the placenta to the mother, but the resultant antibodies can return to the foal only via the colostrum, whereas in women similar antibodies may traverse the placenta and cause an antigen–antibody reaction in the unborn fetus.

There is good evidence that both the microscopic and macroscopic structure of the placenta may be influenced by factors such as the nutritional status of the dam during pregnancy and breed. The consequences that such factors may have for impaired fetal and postnatal growth of large domestic animals have been extensively reviewed by Wu et al. (2006). The following examples may illustrate this: A comparison of the gross morphology of the placentomes of ewes subjected to modest undernutrition during the first 70 days of pregnancy with those on a normal control diet has shown a change in the distribution of the types of placentomes based on the classification of Vatnick et al. (1991). Undernutrition of the dam results in a decrease in the type A placentome (the 'classical' shape) and an increase in the type D, in which the fetal component of the placenta is increased (Steyn et al. 2001). This shows that the placenta is a dynamic organ and may respond to the demands of the fetus – in this case the provision of sufficient nutrition to maintain normal intrauterine growth. However, there is also evidence in sheep that the response of the placentomes

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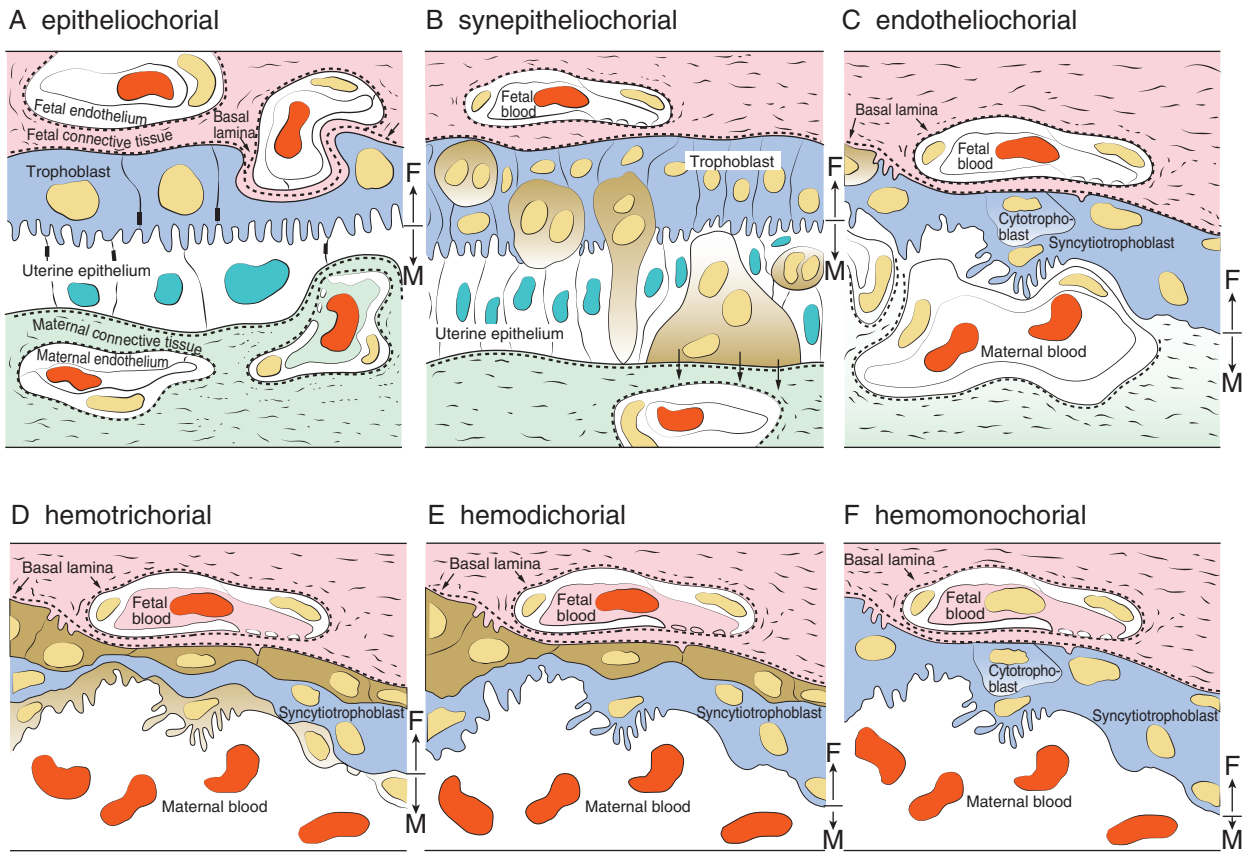

• **Fig. 4.16** Diagrammatic representation of the maternofetal barrier in placentas according to the classification of Grosser (1909) and expanded by Enders (1965). (From Leiser & Kaufmann 1994.) Tissues on the fetal side are indicated by *F*, those on the maternal side by *M*. See text for further explanation.

to undernutrition in midgestation can be influenced by previous nutritional conditioning (Vonnahme et al. 2006). On the other hand, 'overnutrition' of pregnant adolescent sheep (in order to promote rapid growth of these young ewes) also impaired vascular development in the fetal portion of the placentomes during early to midpregnancy and resulted in reduced fetal weight (Redmer et al. 2009). Apparently, nutritional benefits in this model were directed to the mother at the expense of placental and fetal development.

During the past two decades an overwhelming amount of evidence, obtained from studies in humans, laboratory animals, and farm animals, has shown that the intrauterine environment of the conceptus (i.e., fetus and placenta) may alter the expression of the fetal genome during prenatal development. This so-called 'fetal programming' may result in permanent changes in the morphology, physiology, and metabolism of the offspring and may thus have consequences for health, production parameters, and diseases during later life. For further reading on this topic, one is referred to the pioneering book by Gluckman and Hanson (2005) and to selected reviews (Fowden & Forhead 2004, Wu et al. 2006, Evans et al. 2016, Fowden et al. 2016, Gonzalez-Bulnes et al. 2016, Symonds et al. 2016).

Fetal Fluids

Cattle

The total quantity of fetal fluid of cattle increases progressively throughout pregnancy; early ultrasonographic pregnancy diagnosis is based on the recognition of allantoic fluid within the uterine horns (see Chapter 5). Its volume averages about 5 litres at 5 months, and 20 litres at term. Sharp rises in the total quantity occur between 40 and 65 days, between 3 and 4 months, and again between 6.5 and 7.5 months. The first and last of these are due to an increase in allantoic fluid, and the second to an increase in amniotic fluid. For nearly the whole of the first third of pregnancy, when the conceptus consists of an elongated allantochorion with a central spheroidal amnion closely investing the relatively small embryo, there is more allantoic fluid; during most of the second third of pregnancy, amniotic fluid predominates, but for the greater part of the final third, allantoic fluid is again clearly in excess (Fig. 4.17).

Throughout gestation the allantoic fluid is watery or urine-like. In the first two-thirds of pregnancy the amniotic fluid is similar, but for the remainder of gestation it is a mucoid fluid. The latter change gives it the lubricant property that is so helpful at parturition. At birth the allantoic sac forms the first and the amnion the second 'water bag'. The allantochorion is thicker and tougher than the transparent amnion.

Sheep

The studies of Malan and Curson (1937) and Cloete (1939) have revealed that the total volume of fetal fluid increases with advancing age of the conceptus but that the separate fetal fluid volumes show different tendencies. As in the cow, the visualisation of allantoic fluid at the end of the first month can be used as a tool for early pregnancy diagnosis (see Chapter 5). During the first 3 months, apart from an initial preponderance, the allantoic fluid accumulates

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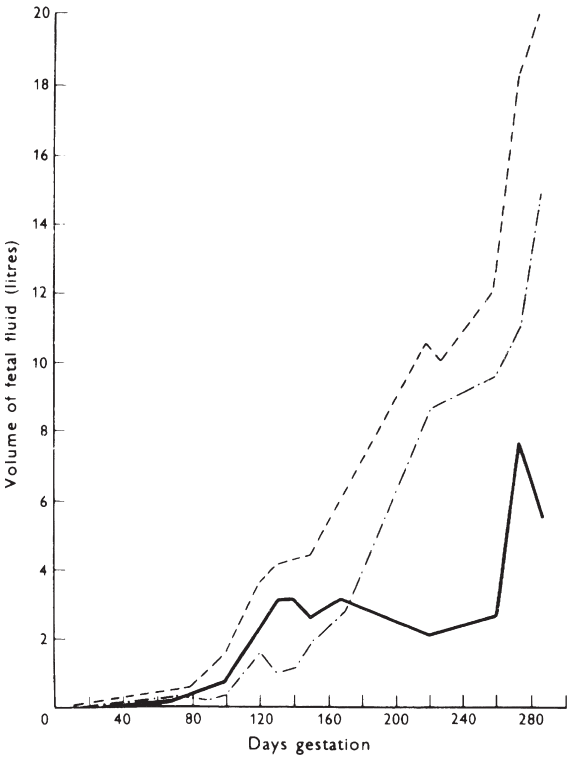

• **Fig. 4.17** Volume of fetal fluids at successive stages of pregnancy in the cow: dashes, total; dashes and dots, allantoic; continuous line, amniotic. (From Arthur 1969.)

TABLE 4.1 Volume of fetal fluids in the sheep (mL)

Month of Gestation	Amniotic	Allantoic	Total
1	3	38	41
2	169	89	258
3	604	131	735
4	686	485	1171
5	369	834	1203

(Data from Malan & Curson 1937 and Cloete 1939.)

slowly, e.g., 131 mL at 3 months, whereas the increase in amniotic fluid occurs largely during this time, so that at 3 months it reaches 604 mL. In the fourth month the rate of increase in allantoic fluid greatly accelerates to 485 mL whereas the amniotic fluid increases only slightly. During the last (fifth) month of gestation, the allantoic fluid almost doubles its volume to 834 mL, but the volume of amniotic fluid diminishes to 369 mL (Table 4.1). When twins are present, the totals of fluids are approximately doubled (Arthur 1956), and the decrease of amniotic fluid apparently starts already during the third month (Naaktgeboren & Stegeman 1969).

Horse

It is recalled that the first fluid to be recognised during early ultrasonographic pregnancy diagnosis (from about day 10 onwards) is the fluid within the yolk sac. The allantoic fluid becomes visible after about day 24 (Ginther 1995). Ranges of weights of the total fetal fluid for each month of pregnancy in the mare as given by Rűsse and Grunert (1993) are shown in Table 4.2.

TABLE 4.2 Weight of fetal fluids in the horse

Month of Gestation	Weight of Total Fluid (kg)
1	0.03–0.04
2	0.3–0.5
3	1.2–3.0
4	3.0–4.0
5	5.0–8.0
6	6.0–10.0
7	6.0–10.0
8	6.0–12.0
9	8.0–12.0
10	10.0–20.0
11	10.0–20.0

TABLE 4.3 Volume of fetal fluids in the horse

Estimated Gestation (months)	Fetal Body Length (cm)	Amniotic (mL)	Allantoic (mL)
3	10	30[a]	2300
4.5	20	100	4090
6	44	6200	4090
	77	3700	4800[b]
9	80	9200	—
	81.5	1670	5210

[a]Estimated volume in a preserved specimen
[b]Some allantoic fluid lost in transit

Serial values for the separate amounts of amniotic and allantoic fluids are only available for a limited number of pony mares during the first 200 days of gestation (Ginther 1992). The data showed that amniotic fluid volume remains smaller than allantoic fluid volume but increased rapidly between days 100 and 160 to equal the volume of allantoic fluid at midpregnancy. The observations of Arthur (1956) in mares (Table 4.3), together with data provided by Amoroso (1952), Zietzschmann (1924), and Ginther (1992), suggest that the trends are similar to those of cattle. Thus allantoic fluid predominates in early as well as late pregnancy and measures 8 to 15 litres at term. The amniotic fluid volume differs from that of cattle. It is low during the first 3 months; thereafter it increases more rapidly and at midpregnancy equals the volume of allantoic fluid, whereas at term it is 3 to 5 litres.

Pig

The volume of allantoic fluid shows an early rise to about 200 mL at 30 days and enables an easy recognition during early ultrasonographic pregnancy detection (see Chapter 5). Thereafter, it

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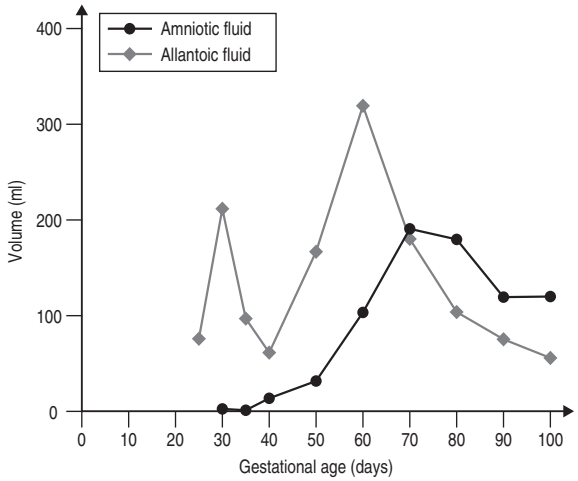

• **Fig. 4.18** Mean changes in the volume of amniotic and allantoic fluid in porcine conceptuses. (From Knight et al. 1977.)

declines and then increases to reach a maximum of 320 mL at 60 days, before gradually declining to term. Amniotic fluid gradually increases, reaching a maximum of about 200 mL on day 70, before declining slightly until term (Fig. 4.18).

Dog and Cat

Published serial values for the separate amounts of amniotic and allantoic fluids are not available for the dog and cat. In kittens of fetal body length up to 9 cm, the amniotic fluid increases gradually to 10 to 15 mL, after which there is some decrease, followed by a slight rise just before term. The allantoic fluid starts with a more rapid rise and at midterm is higher than the amniotic (20 mL), but towards the end of gestation declines to about 6 mL.

Form and Disposition of the Fetal Sacs

Ruminant Species

Throughout gestation the amnion enclosing the fetus, together with the larger portion of the allantochorion, remains in the uterine horn adjacent to the ovary with the corpus luteum of pregnancy; a smaller 'limb' of allantochorion projects across the uterine body into the other horn. Most of the allantoic fluid gravitates to the poles of the allantochorion, which lie in the dependent parts of the uterine horns, and the uterine distension thus caused in cattle is the chief clinical sign of early pregnancy. By the third month, considerable fluid (up to 0.75 litres) accumulates in the spherical amnion, and it now gives rise to the main palpable mass in the pregnant horn (see Figs 4.5–4.8).

On the inner face of the amnion of ruminants, particularly near and on the umbilicus, are numerous raised, rough, discrete, round foci called amniotic plaques or pearls. They are rich in glycogen but of unknown function and usually disappear after 6 months of gestation. Towards the end of pregnancy, smooth, discoid, rubber-like masses may float in the allantoic fluid. They originate from invaginations of the allantochorion around accumulations of compacted histiotroph between the chorion and endometrium. Initially, they can be found still attached by a stalk to the allantochorion. They are called 'hippomanes' (in cows more rarely 'boomanes') and have no functional significance (Rűsse & Grunert 1993).

Horse

Much of the allantochorion and the greater part of the amnion are contained within the gravid horn, with a direct continuation of similar width into the uterine body. The part of the allantochorion that projects into the nongravid horn is much narrower and is about two-thirds of the length of the gravid horn segment (see Fig. 4.11A). In the rare case of a bicornual pregnancy, the allantochorion occupies both cornua to a similar extent. A hippomanes on the inside of the chorioallantoic membrane of a mare is illustrated in Fig. 4.13.

Pig

After the distribution and spacing of blastocysts at about 10 days of gestation, the rapid elongation of the blastocysts (see later in this chapter) results in their distribution along the length of both uterine horns – from one ovary to the other – and with the one nearest the body of the uterus often extending into both horns. The uterine surface of the sow's allantochorion is studded with small, round, grey foci called 'areolae' in which the placental villi are absent. They occur opposite focal aggregations of uterine glands (Dantzer & Winther 2001).

Relationship Between Fetal Membranes of Twins and Multiple Fetuses

The relationship of the membranes of contiguous fetuses is simplest in carnivora, and although the extremities of the allantochorionic sacs impinge on each other, they remain separate. The next gradation is in the mare pregnant with twins; here, apparently owing to the lack of uterine length, the distal pole of one allantochorion invaginates the proximal extremity of the other. According to Williams (1939), this results in an unequal sharing of the uterine space and thus competition for the reduced area of endometrium available with which the trophoblast can become attached to form the placenta. Thus usually one fetus suffers severe growth retardation, resulting in: death in utero resulting in the birth of the survivor as a singleton; complete failure of the pregnancy with abortion; or the birth of live twins of very disparate size (Figs 4.19A and B).

Two noticeable features of the allantochorionic sacs of the pig are that from about 27 days of gestation, the tips of the elongated allantochorion become ischaemic and then necrotic (Crombie 1971, Flood 1973; see Fig. 4.14A and B). It has been suggested that this might be a mechanism to prevent vascular anastomosis, which might result in the development of freemartins (see Chapter 9). Frequently, the ends of adjacent allantoic sacs become 'glued' together by a gelatinous substance, although the attachment is not a strong one. Furthermore, in some cases there is complete fusion of the allantochorion of adjacent sacs as early as 22 days, although there appears to be no vascular anastomosis thus eliminating the development of freemartins (Crombie 1971). The intervening 'wall' between adjacent conceptuses often disappears, most likely close to term or during farrowing because of propagated uterine contractions. As a result, there develops a continuous allantochorionic 'tube' through which fetuses are seen to pass during parturition (see Chapter 6). These have been called 'synchorial tubes' by Marrable (1969). Interesting breed differences have been identified in pigs with respect to early and late placental development (Ford 1997, Wilson & Ford 2001). In the Yorkshire breed the placentae increase in size and surface area in the last third of gestation, whereas in the Meishan, placental size remains constant in late gestation, although the density of blood vessels at the allantochorionic/endometrial interface increases markedly (Wilson et al. 1998). In addition, it has also been shown that whereas in the Yorkshire if a conceptus dies as late as 40 days, there is acceleration of the growth rate of

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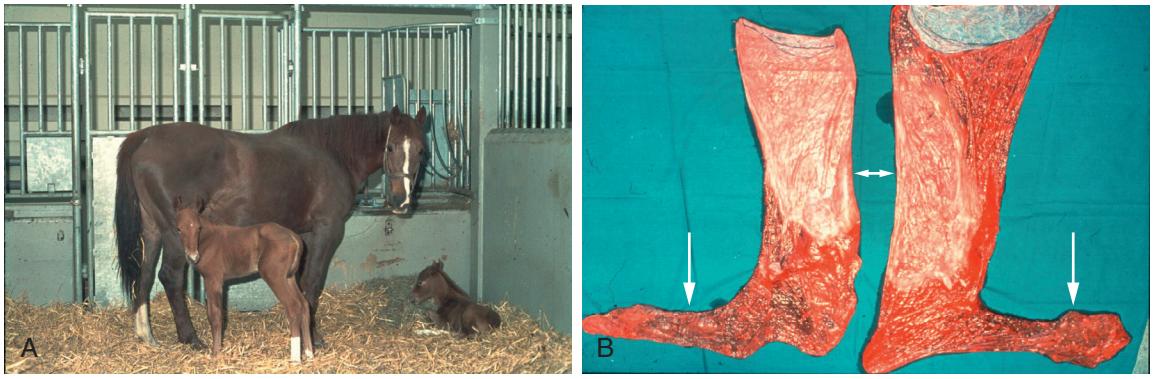
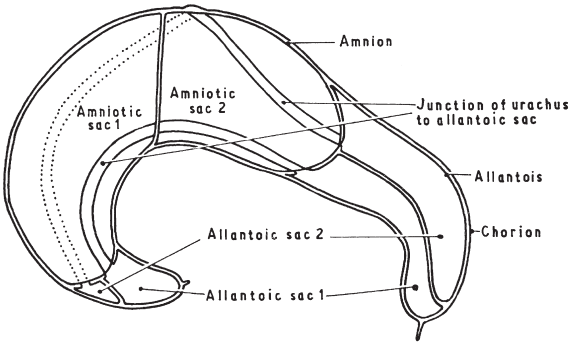
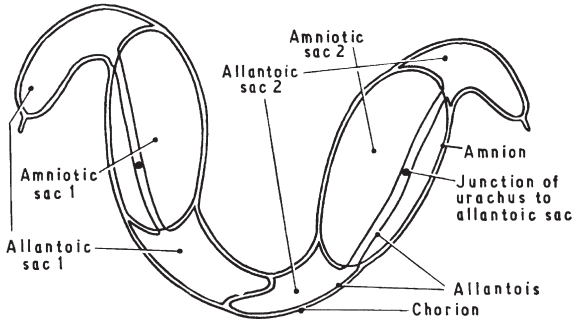


• **Fig. 4.19** (A) Mare with her newborn twins. (B) The collected afterbirths have been inverted so that the chorionic sides of the allantochorionic membranes are exposed. Those parts that were contained within the two uterine horns are at the lower end of the figure *(see arrows)*. Where the two allantochorionic sacs apposed each other, no contact could have been made with the endometrium, and the chorion remained villous (white portions; *double arrow*).



• **Fig. 4.20** Fetal membranes of bovine twins in unicornual gestation. (Courtesy of M. J. Edwards.)



• **Fig. 4.21** Fetal membranes of bovine twins in bicornual gestation. (Courtesy of M. J. Edwards.)

the placentae of the adjacent live ones, in the Meishan this does not occur (Vonnahme et al. 2002). In the rare case of anastomosis of allantoic blood vessels of porcine fetuses of unlike sex, a basis for the 'freemartin' condition exists as in cattle (see Chapter 9).

In the majority of twin or triplet pregnancies of sheep and cattle, contiguous chorionic sacs coalesce (Figs 4.4D, 4.20, and 4.21), and in many cases the allantoic cavities are confluent, whereas in cattle – but only in about 0.8% of sheep – allantoic vascular intercommunication is the rule (see Fig. 4.4D). Such anastomosis, according to Lillie (1917), is present between bovine fetuses from the 40th day, and it forms the main premise of his theory of the origin of the bovine freemartin (see Chapter 9). It is now thought that the passage from male to female fetuses of the anti-Műllerian hormone (rather than androgens) is a crucial factor in causing masculinisation of the female fetus (Padula 2005).

Of 25 sets of equine twins, 11 were found by Vandeplassche et al. (1970) to have blood chimerism, thus indicating vascular anastomosis between the twin placentae. However, on dissection the genital organs of the female members of four heterosexual chimerical pairs were found to be normal, whereas five other mares of heterosexual chimerical pairs were found clinically to have normal genitalia; they had normal oestrous cycles and became pregnant. The fact that blood chimerism has been found in adjacent fetuses of horse, sheep, and pig, as well as cattle gestations, indicates that there is also a basis for the freemartinism in these species. However the observed incidence of freemartinism is very low in pigs and sheep (see Chapter 9) and is nil in horses; this may be due, according to Vandeplassche et al. (1970), to late allantoic vascular anastomosis in these three species compared with the early (30-day) fusion in cattle.

Fetal Movements During Pregnancy

When discussing placental relationships, it is instructive to consider the subject of fetal mobility within the uterus. With the introduction of real-time ultrasonography, direct observations and quantification of (early) fetal movements could be made in domestic animals (Ginther 1995 & 1998, Cohen 1999). The earliest fetal movements in cattle have been observed around day 40, and their frequency rapidly increases thereafter, coinciding with a rapid increase of amniotic fluid. Obviously, in all species, within the amnion, fetal movement about both the longitudinal and transverse axes is possible. Rotation about the former is limited by the length of amniotic sac and the umbilical cord, and about the latter by the length of the fetus and the width of the amnion. In cattle not more than a three-fourths revolution around the long axis is possible and, although several rotations about the transverse axis may occur, a complete rotation of the bovine umbilical cord is not normal; coincidently, it has been seen only in mummified fetuses. In equine fetuses, however, complete rotation of the (amniotic portion) of the umbilical cord is possible. Another possibility for intrauterine fetal movement is the mobility of the entire amniotic sac (with contained fetus) within the allantochorion sac. Owing to

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TABLE 4.4 Pregnant equine uteri: disposition of fetus

Months of Gestation	No. of Cases	Anterior Presentation	Posterior Presentation	Right Horn Pregnant	Left Horn Pregnant
2–3.5	4	2	2	2	2
3.5–4.5	12	7	5	6	6
4.5–5.5	9	4	5	6	3
5.5–6.5	16	9	7	11	5
6.5–7.5	12	8	4	7	5
7.5–8.5	11	9	2	8	3
8.5–10	4	4	0	3	1
10.5–11	4	4	0	3	1
11–12	3	3	0	2	1
Total	75	60	25	48	27
				64%	36%
10	1	Transverse presentation		Bicornual pregnancy	
5	1			Twin pregnancy	

the extensive fusion of the allantochorion to the allanto–amnion in the cow, ewe, and sow, such mobility would be impossible. However, in the mare, such movements do take place and lead to twisting of the allantoic portion of the umbilical cord.

Active fetal movements are responsible for changes in intrauterine presentation, position, and posture during pregnancy and at birth (see Chapter 11). Data collected from pregnant bovine uteri by Naaktgeboren (1963) and Ginther (1998) and from equine specimens by Vandeplassche (1957) (Table 4.4) indicates that, initially, anterior and posterior presentations occur in about equal numbers. At between 5 and 6 months of pregnancy, the body length of the fetal calf exceeds the width of the amnion, and thus at this stage the final polarity of 95% of fetuses in anterior presentation is adopted. Based on weekly examinations in a group of 10 mares, some 40% to 60% of fetuses were still in posterior presentation up to the end of the fifth month. The final gestational presentation of nearly 99% of foals, anteriorly disposed, occurred from the seventh month onwards, with few changes thereafter (Ginther 1993). It would seem likely that changes of the longitudinal presentation that occur during gestation in the mare are due to movements of the amnion within the allantochorion, initiated and supported by active fetal movements. An explanation for the final overwhelming proportion of anterior presentations in the cow was given by Naaktgeboren (1963). By submerging and measuring of fetuses of different gestational ages in a water bath, it was concluded that the point of gravity of their body shifted caudally. Together with the expansion of the amniotic cavity towards the tip of the pregnant horn, this enables the fetus to descend with its hindquarters in the horn and assume an anterior presentation. In addition, the hindlimbs may become trapped behind the sharp kink of the pregnant horn at the curvatura minor, which contributes to stabilising the fetus in anterior presentation (Naaktgeboren & Slijper 1970). Similar mechanisms appear to play a role in other domestic ruminants and in mares. Both in the pig (Taverne et al. 1977) and dog (Van der Weyden et al. 1981), some 20% (pig) and 7% (dog) of the fetuses were born in a presentation that differed from the one observed during laparotomy a few weeks earlier. Yet there is still no explanation for the slight preponderance of anterior presentations observed during late pregnancy and parturition in species such as the pig, dog, and cat.

Fetal Growth and Size

Fetal growth depends on many genetic and environmental factors. Given the variety of (neonatal) body sizes found in different breeds of the same domestic species, fetal growth curves have to be judged with caution. Yet it is sometimes necessary to estimate the age of aborted fetuses; for example, when details of their origin are unknown. For this purpose, growth curves, based on measurements of embryos/fetuses obtained at the slaughterhouse (Table 5.3) or from laparotomies at different stages of gestation (bovine fetus: Figs 4.22 and porcine fetus: 4.23) may be useful. Richardson et al. (1976) have shown that long bone length (conveniently radius and tibia) is a reliable indicator of fetal age from 50 days of gestation to term in the sheep and may be obtained radiographically in the living fetus or by postmortem measurement. Mean values for the respective ages of sheep fetuses are shown in Table 4.5. The occurrence and implications of fetal growth retardation in domestic ruminants, pigs, and horses have been reviewed by Wu et al. (2006).

During the past few decades, many studies using B-Mode real time ultrasonography have demonstrated that it is possible to measure a variety of fetal dimensions, thereby enabling ageing of fetus(es). Published data are available for horses (Ginther 1995, Hendriks et al. 2009), cows (Kähn 1989, Ginther 1998, Breukelman et al. 2004, Chavatte-Palmer et al. 2006), sheep (Russell 1989, Aiumlamai et al. 1992), and goats (Medan et al. 2004, Karen et al. 2009). In dogs (Moriyoshi et al. 1996, Groppetti et al. 2015, Socha et al. 2015) and cats (Zambelli et al. 2002, 2004) such data have also been used for the prediction of the parturition date.

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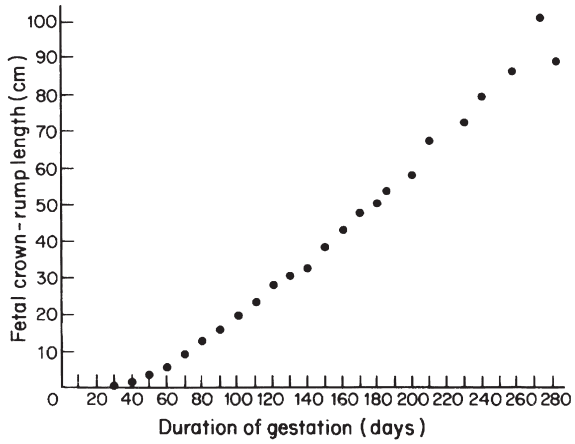
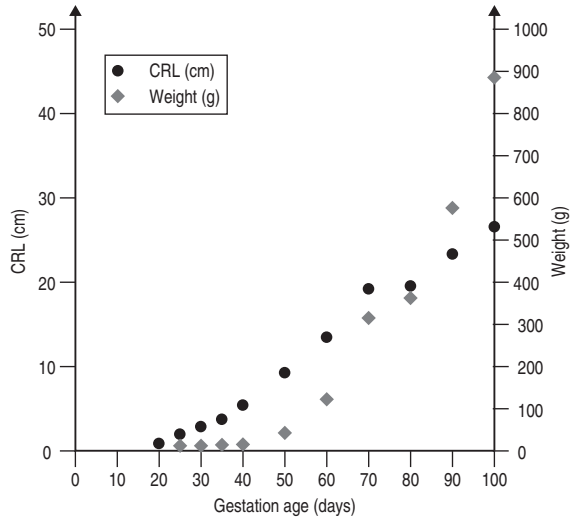


• **Fig. 4.22** The growth curve of the bovine fetus.



• **Fig. 4.23** Changes in the mean +/- standard error of mean (sem) of crown–rump length (CRL) and weight of porcine fetuses. (From Knight et al. 1977.)

TABLE 4.5 Length of ovine fetal long bones at various stages of conception

Days After Conception	Length of Radius (mm)	Length of Tibia (mm)
50	4.8	5.0
60	10	12
70	16	19
85	25	32
100	36	47
110	47	63
120	56	76
130	67	91
140	74	100
150	79	107

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5

Pregnancy and Its Diagnosis

MARCEL TAVERNE AND DAVID E. NOAKES

Maternal Recognition of Pregnancy

In most domestic species, the establishment and maintenance of pregnancy require that the luteal phase of the oestrous cycle is prolonged by the persistence of a single corpus luteum (CL) or a number of corpora lutea (CLs). As a result of the persistence of the luteal tissue, progesterone concentrations remain elevated. This results in a negative feedback on the hypothalamus and anterior pituitary with a resultant inhibition of follicular development and ovulation and, in polyoestrous species, a prevention of return to oestrus. In many species the placenta subsequently replaces or supplements the luteal source of progesterone.

In Chapter 1 the importance of the CL in regulating the periodicity of the oestrous cycle was discussed, and the role of prostaglandin (PG)$F_{2\alpha}$, produced by the endometrium, in causing regression of the CL and the consequent return to oestrus was described. The presence of a viable, developing embryo(s), however, prevents the CL from regressing and thus in polyoestrous species inhibits the return to oestrus. This phenomenon was described by Short (1969) as the 'maternal recognition of pregnancy'. It is particularly interesting because this maternal endocrine response is detectable before the blastocyst is attached to the endometrium by microvilli. In five of the domestic species the time of maternal recognition of pregnancy has been determined (Table 5.1). Mechanisms involved in the maternal recognition of pregnancy in domestic species have been thoroughly reviewed (Spencer & Bazer 2004, Weems et al. 2006, Bazer 2013) and clearly illustrated (Senger 2005, Bazer 2013). Knowledge of this topic is essential for understanding the possible background of pregnancy failures that are based on disruption of these early communication mechanisms between embryo and dam. In addition, these mechanisms might provide a means for the development of tests for the early diagnosis of pregnancy.

Sheep

In sheep early work by Moor (1968) and Martal et al. (1979) demonstrated that the conceptus produces a protein. In recent years it has been characterised as existing in three or four isoforms of molecular weight of 19 to 24 kDa, depending on glycosylation. Originally, it was named ovine trophoblast protein or oTP-1. This substance has been shown to be a type 1 interferon, classified as an ovine tau interferon (oIFN-tau), and is nonglycosylated. A similar but N-glycosylated protein, bovine tau interferon (bIFN-tau) has been found in cows (see later in this chapter). It is produced by the trophectoderm from about day 10, at the time that the blastocyst starts to elongate (Chapter 4).

Details of the mechanism of luteolysis have been described in Chapter 1. The effect of IFN-tau in the maternal recognition of pregnancy is to alter the dynamics of $PGF_{2\alpha}$ secretion at this early stage of pregnancy, compared with the same stage of the oestrous cycle. The number of luteolytic pulses is reduced between days 15 and 17, yet surprisingly the basal secretion of $PGF_{2\alpha}$ is increased at this time (Zarco et al. 1988). A similar phenomenon was observed during spontaneous prolongation of the luteal phase in unmated cyclic ewes (Zarco et al. 1984). IFN-tau secreted by the embryo silences the transcription of the oestrogen receptor alpha gene in uterine luminal and superficial glandular epithelial cells that precedes the oestrogen-induced expression of oxytocin receptors in these cells. The consequence is that IFN-tau indirectly suppresses the expression of oxytocin receptors in endometrial epithelial cells. Oxytocin of luteal and pituitary origin can thus no longer bind to the uterus, and this results in a reduction in the pulsatile secretion of $PGF_{2\alpha}$ (Spencer & Bazer 2004; Bazer 2013). Because basal production of $PGF_{2\alpha}$ is even greater in pregnant than in cyclic sheep, and because IFN-tau does not affect expression of cyclo-oxygenase 2 in the endometrium of early pregnant sheep, other researchers have emphasised a reduced luteal sensitivity to $PGF_{2\alpha}$ and enhanced production of luteotrophic PGE_2 as the dominant mechanisms in the maternal recognition of pregnancy in this species (Costine et al. 2007). There is indeed recent evidence that oIFN-tau may protect the ovine CL from luteolytic actions of PGF by an endocrine route (Antoniazzi et al. 2013; Romero et al. 2015).

Cattle

In the cow the importance of the blastocyst in prolonging the life span of the CL was shown by the studies of Northey and French (1980). They found that, if the blastocyst was removed at day 17 or day 19, the interoestrous intervals were extended to 25 and 26 days respectively, compared with those in which the embryo was removed at day 13, or that were not mated in the latter cases the intervals were 20 to 21 days. The antiluteolytic signal produced by the bovine conceptus is a protein with a molecular weight of 24,000. Originally called bovine trophoblast protein (bTP-1), it was found to cross-react immunologically with oTP-1. As well as having a high amino acid sequence homology with both oTP-1 and IFN-alpha, it also possesses antiviral activity (Bazer et al. 1991). As in sheep, it is now classified as tau interferon (bIFN-tau), with maximum secretion occurring between days 16 and 19 of gestation. It is first secreted at the time of elongation of the blastocyst and, unlike oIFN-tau, continues to be secreted until day 38 of gestation

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TABLE 5.1 Time of maternal recognition of pregnancy

Species	Day of Maternal Recognition of Pregnancy	Day of Definite Attachment
Sow	12	18
Ewe	12–13	16
Cow	16–17	18–22
Mare	12–16	36–38
Goat	17	

(After Findlay 1981.)

(Bartol et al. 1985, Godkin et al. 1988). When it is infused into the uterine lumen of nonpregnant cyclical cows between days 14 and 17, the life span of the CL is extended. Similar results have been obtained following the administration of recombinant bovine INF-alpha$_1$ using the same route of administration and also intramuscularly (Plante et al. 1989).

As in the ewe, the luteolytic patterns of $PGF_{2\alpha}$ are attenuated in the cow in early pregnancy; however, the luteolytic pulses are abolished without major increases in basal secretion (Thatcher et al. 1995). Similar to the effect of oIFN-tau in sheep, bIFN-tau exerts this antiluteolytic effect by silencing the expression of the oestrogen receptor in uterine epithelial cells, thereby preventing an oestrogen-induced expression of oxytocin-receptors in these cells. It is important to realise that, besides the production of bIFN-tau, the preimplantation embryo produces a large number of unique proteins that were found in the uterine luminal fluid during the pregnancy recognition period (day 16–18; Forde et al. 2015). Together with bIFN-tau and certainly also due to the actions of bIFN-tau, these proteins play a crucial role in the interactions between preimplantation embryo and endometrium to facilitate conceptus growth and development.

Goat

In the goat, the removal of conceptuses from the uterine lumen between days 13 and 15 does not prolong the life span of the CL, but removal on day 17 increases the interoestrous interval by 7 to 10 days. The caprine conceptus secretes a protein originally designated cTP-1, which, as in other ruminants, is cIFN-tau (Gnatek et al. 1989). Caprine IFN-tau is mixture of nonglycosylated and N-glycolylated forms and is secreted between days 16 and 21 of gestation. As has been shown in sheep and cattle, this protein suppresses pulsatile prostaglandin secretion from the endometrium and extends the lifespan of the CL (Newton et al. 1996). This prolongation of luteal function apparently also occurs spontaneously in pseudopregnancy in this species, when in unmated does the CL persists and fluid (hydrometra) accumulates in the uterine lumen (Pieterse & Taverne 1986). Active immunisation of cyclic goats against $PGF_{2\alpha}$ results in luteal persistence and development of hydrometra (Kornalijnslijper et al. 1997).

Pig

Although in the sow the maternal recognition of pregnancy also involves the prevention of luteolysis by uterine-derived prostaglandins, the mechanisms are quite different from those described previously for the domestic ruminant species. After their entry into the uterus on day 4, some 2 days after ovulation, embryos start migrating throughout both uterine horns so that by day 12 they have more or less become evenly distributed, a process called 'spacing'. The early porcine conceptus has been shown to convert progesterone to oestrone and oestradiol-17beta, as well as another isomer of 16alpha, 17-oestradiol (Fischer et al. 1985). The production of oestrogens increases with the rapid elongation of the blastocyst, which may reach a length of 80 to 100 cm each between days 12 and 15. As a result of the spacing and elongation, the conceptuses are able to influence locally a large surface of the endometrium. The oestrogen production by the conceptus plays a vital role in the maternal recognition of pregnancy and the extension of the life span of the CLs. The administration of exogenous oestrogens parenterally from day 12 in nonpregnant gilts has been long known to be luteotrophic, thus prolonging the life span of the CLs and extending the interoestrous interval (Kidder et al. 1955). In addition, a wide range of maternal (endometrial) and conceptus-derived products, such as proteases, calcium, growth factors, adhesion molecules and cytokines, are involved in a complex signalling cascade to establish pregnancy, as well as attachment and implantation (Jaeger et al. 2001). After the initial secretion of oestrogens at day 11, a second sustained release of oestrogens by the conceptus is necessary between days 14 and 18 for luteal persistence beyond day 25 (Geisert et al. 1990). Several mechanisms have been proposed by which the conceptus-secreted oestrogens may prevent luteal regression (Ziecik 2002). These include a direct luteotrophic effect, a reduction in the endometrial synthesis and a release of $PGF_{2\alpha}$. The luteotrophic effects include maintenance of LH receptors in the CLs and secretion of prostaglandin E_2 to protect the CLs against the luteolytic action of $PGF_{2\alpha}$. However, there is convincing evidence that they exert their effect by altering the transport of $PGF_{2\alpha}$ from an endocrine (towards the uterine vasculature) to an exocrine (into the uterine lumen) direction, thereby preventing $PGF_{2\alpha}$ from reaching the CLs (Bazer et al. 1984; Bazer, 2013). Oestrogen-induced prolactin receptors in the endometrium might facilitate this redirecting. The fate of the intrauterine $PGF_{2\alpha}$ is not known; however, fetal membranes readily metabolise it to PGFM (15-keto-13, 14-dihydro-$PGF_{2\alpha}$), which is inactive. The oestrogen stimulation of calcium secretion into the uterine lumen also appears to be involved in the process.

As in ruminant species, the pig conceptus produces interferons at the time of elongation (11–17 days of gestation) (Cross & Roberts 1989); to date, two interferons have been identified, a type I (IFN-delta) and a type II (IFN-gamma). The infusion of total conceptus-derived secretory proteins into the uterine lumen failed to prolong the life span of the CLs in cyclic sows (Harney & Bazer 1989). Their precise functions are not known, but it has been postulated that they may have a specific protective antiviral role or that they may act in conjunction with conceptus-derived oestrogens in prolonging the life span of the CLs (La Bonnardiegravere 1993, Cencic & La Bonnadiegravere 2002).

Horse

In the mare the mechanisms responsible for the recognition of pregnancy are less completely understood (Allen 2005, Stout 2016). Evidence of the importance of the developing conceptus in this process has been shown by the effects of its removal at varying stages of gestation: if it was removed at 10, 15 and 20 days, then the return to oestrus was 22.3, 38.0 and 47 days respectively (Hershman & Douglas 1979). However, it is not clear yet how the

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early embryo prevents luteolysis in the mare (Stout 2016). A low molecular-weight protein has been identified in the uterine flushings of mares in dioestrus, which persists during pregnancy (McDowell et al. 1982). The equine conceptus also produces oestradiol and oestrone (Berg & Ginther 1978, Zavy et al. 1979), which enhance the production of a glycoprotein, uteroferrin, by the uterus. PGF secretion from the pregnant uterus is apparently blocked because lower concentrations of PGF have been identified in the uterine venous blood of pregnant mares compared with nonpregnant mares (Douglas & Ginther 1976) and similarly lower concentrations of PGFM in the peripheral circulation (Kindahl et al. 1982). There does not appear to be any sequestration of PGF in the uterine lumen during early pregnancy, but when endometrial tissue was incubated in vitro with material of conceptus origin, there was a reduction in PGF synthesis or secretion (Berglund et al. 1982). It has been demonstrated that this suppression of uterine PGF production is caused by a temporary (until about day 16) conceptus-directed downregulation of endometrial prostaglandin synthase 2 activity, accompanied by a posttranscriptional inhibition of endometrial oxytocin-receptor expression. As a result, the endometrium becomes nonresponsive to oxytocin of endometrial and hypothalamic origin (see review by Stout 2016). An important feature of the mechanism of maternal recognition of pregnancy in the horse is the migration of the spherical conceptus within the uterine lumen until it becomes 'fixed' at 16 to 18 days of gestation at the base of the uterine horn. This has been demonstrated in some elegant experiments by McDowell et al. (1988). By restricting the mobility of the conceptus using ligatures at various parts of the uterus, the maternal recognition was compromised so that the CL regressed spontaneously. Migration of the conceptus appears to be caused by uterine contractions induced by prostaglandins either from a conceptus or uterine origin (Stout & Allen 2001). Unlike in ruminants, the equine conceptus does not secrete interferon, and although it produces oestrogens in high amounts from day 10 onwards, oestrogens appeared unable to extend the luteal lifespan when applied in physiological doses (see review by Aurich & Budik 2015). However, as already mentioned, it does produce a variety of other hormones and growth factors that may indirectly be involved in maternal recognition of pregnancy. Yet the exact nature of the antiluteolytic factor(s) secreted by the conceptus remains to be elucidated.

Dog

The bitch is atypical in that the length of the luteal phase (metoestrus/dioestrus) is not shorter, but frequently exceeds that of pregnancy. No increases in PGFM concentrations have been found during the demise of the CLs in nonpregnant dogs (Luz et al. 2006; Concannon 2009), and surgical removal of the nonpregnant uterus in metoestrus/dioestrus does not prevent the demise of the CLs (Okkens et al. 1985). So there appears to be no need for maternal recognition of pregnancy to maintain the life span of the CLs and the continuation of progesterone production. However, it may seem a contradiction that CLs of both the pregnant and nonpregnant bitch require luteotrophic support by LH and, predominantly, prolactin, especially during the second half of their life span (Onclin et al. 2000; Kowalewski 2014).

Cat

The length of the luteal phase in the mated but nonpregnant (pseudopregnant) queen is about 40 days, whereas in pregnant females luteal progesterone production continues for another 20 days. Little is known about the mechanisms underlying the maternal recognition of pregnancy in the queen. In cats with hormonally induced corpora lutea, neither hysterectomy nor surgical diversion of uterine venous blood away from the ovarian vasculature had any effect on the duration of the luteal phase (Wheeler et al. 1988). This suggests that, as in dogs, maternal recognition of pregnancy in cats does not involve prevention of luteolysis caused by a uterine luteolysin.

Pregnancy Diagnosis

The detection of pregnancy, or its absence, in domestic species is important in a number of respects, but particularly in relation to their husbandry. In polyoestrous species (cattle, horse, pigs, sheep, goats) early and accurate detection of the nonpregnant individual is important because it enables early rebreeding. In recent years many new biochemical markers of pregnancy have been identified, and although they can be very accurate, often they can only be detected in a laboratory using expensive equipment and by skilled scientists. Particularly in farm animal production systems, it is important that a positive or negative diagnosis is made at the time the animal is being examined.

The sensitivity of a diagnostic method refers to the correctness of identifying pregnant animals, whereas the specificity refers to the correctness of identifying nonpregnant animals. However, it is important that inexperienced veterinarians should select a time when they are confident of their accuracy in making the diagnosis, irrespective of the method used.

In the mid-1970s it was observed that lymphocytes retrieved from mice shortly after mating displayed altered reactivity during rosette inhibition testing (Morton et al. 1974). During rosette inhibition testing, lymphocytes are incubated with heterologous erythrocytes and form 'rosettes', which describes the arrangement of erythrocytes around a central lymphocyte. The inhibition of rosette formation observed in females during early pregnancy is attributed to the presence of an immunosuppressive protein, the 'early pregnancy factor' (EPF), now identified as chaperonin 10 or heat-shock protein10. Early pregnancy factor is undoubtedly the earliest sign of the developing embryo affecting the maternal environment; in the horse it can be identified as early as 48 hours after mating. In addition to the rat its presence has also been detected in pig and cow. Thus it enabled the very early identification of individual animals in which fertilisation had not occurred and thus could be prepared for early rebreeding. Kits were available commercially to perform the rosette inhibition test on the farm or breeding establishment, but unfortunately they were not reliable.

From a practical perspective, in a nonpolyoestrous species such as the dog, early detection is not as critical. Early identification of nonpregnant individuals enables decisions to be made on the future of individual animals. In the case of nonpregnant individuals this could be the choice between rebreeding and culling; for pregnant individuals it would enable selecting the time of weaning or applying nutritional management.

A variety of methods can be used to detect pregnancy; many are common to all species whereas others are species-specific. They can be considered under four broad categories: management, clinical, ultrasonographic, and laboratory-based. Of these the first two are generally well tried and tested, whereas the latter two are generally more specific, and in most cases have been improved and refined extensively in recent years. Because ultrasonography is now used

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in all domestic species, the broad principles behind the technology ultrasonography will be reviewed before the methods applicable to the various species are described.

Ultrasonography

Three types of ultrasound technique have been used for pregnancy diagnosis. The ultrasonic fetal pulse detector was the first type used. This is based upon the Doppler phenomenon, in which high-frequency (ultrasonic) sound waves emitted from a probe placed on the exterior of an animal, or in the rectum, are reflected at an altered frequency when they strike a moving object or particles, e.g., the fetal heart, fetal body parts or blood flowing in fetal or uterine arteries. The reflected waves are received by the same probe, and the differences in frequencies are converted into amplified audible sound or converted into a colour image; this is particularly useful in measuring blood flow in both arteries and veins.

The ultrasonic amplitude depth analyser (A-mode) relies upon a transducer head that emits high frequency sound waves and receives the sound reflected at interfaces of tissues of different acoustic impedance. The echoes are shown as a one-dimensional display of echo amplitudes for various depths, usually on an oscilloscope, but also on the newer light-emitting diodes or by 'squeak-producing' devices. This has been used successfully in many species, notably the sow.

A more advanced ultrasound technology is the one that uses the B (brightness) mode image display of the acoustic impedance, providing a two-dimensional (cross) section of the tissues. The technique is frequently referred to as real-time ultrasound or imaging. This just implies that there are live or moving displays in which the echoes are recorded continuously. It has become a very versatile tool in studying a wide range of reproductive events in many species. The transducer probe can be inserted into the rectum or vagina or placed on the surface of the body. The transducer contains numbers of piezoelectric crystals, which, when subjected to an electric current, expand or contract and produce high frequency sound waves. When these sound waves are transmitted through tissues, a proportion, depending upon the characteristics of the tissue, will be reflected back to the transducer. The returning echoes will compress the same piezo crystals, resulting in the production of electric impulses, which are displayed in B-mode as a two-dimensional display of dots on a screen. The brightness of the dots will be proportional to the amplitude of the returning echoes and hence will provide an image ranging from black through various shades of grey to white. In the linear transducers the piezo crystals are arranged in a number of parallel elements, whereas in the sector the ultrasound beam is produced by a single crystal, which oscillates or rotates to produce a fan-shaped beam. The piezo crystals rotate through an angle, producing wedge-shaped image. An intermediate form is the so-called curved array transducer, which in fact is a modified linear array with a curved surface. Liquids, if clear, do not reflect ultrasound and thus are depicted as black on the screen, i.e., nonechogenic, whereas at interfaces of solid tissues such as bone or cartilage, a high proportion of the sound waves is reflected, i.e., they are echogenic and appear white on the screen. Each transducer produces ultrasonic waves at frequencies between 1 and 18 MHz. The most commonly used frequencies are 3.5, 5 and, more recently, 7.5 MHz. The lower frequency transducers give better tissue penetration but poorer resolution. Because when using the transrectal approach the structures requiring imaging are within a few centimetres of the transducer head, high frequency equipment is the most effective. So the choice of the shape and frequency of the transducer mainly depends on the type of investigation to be performed.

Because a tissue/gas interface can result in up to 99% of the sound waves being reflected, it is important that air should not be trapped between the transducer face and the tissues to be examined. For this reason a coupling medium or gel (usually methyl cellulose) is applied to the transducer face before it is placed on the skin or rectal mucosa so that air is eliminated. It is also important to select an area that is relatively hairless, or alternatively, it may be necessary to clip the hair. Curved-array and sector transducers allow ready access to most of the thoracic and abdominal viscera, although very superficial structures may not be readily identified because of the shape of the beam. Sector and curved-array transducers require less skin surface contact, which can reduce the time required to examine each animal; hence they are used for the transabdominal approach, especially in sheep. Linear transducers are usually cheaper to buy and more robust, and they produce a rectangular image, which is easier to interpret. The transducer should be small enough to be cupped in the hand, smooth in contour, waterproof, and easy to clean (Boyd & Omran 1991). For good biosecurity the transducer should be thoroughly cleaned, especially when the same equipment is used on different farms.

Readers who wish to extend their knowledge of these techniques are recommended to consult Ginther (1995; 2014), Taverne and Willemse (1989) and the textbooks by Kähn et al. (1994) and Goddard (1995), covering the major applications in reproduction of domestic species.

Pregnancy and Its Detection in the Horse

Endocrinology

The endocrine changes in the mare during pregnancy are particularly unusual when compared with other domestic species because of the development of temporary hormone-producing structures, the endometrial cups (see Chapter 4).

After ovulation and the formation of the corpus haemorrhagicum and the CL, plasma progesterone concentrations in the peripheral plasma rise to 7 to 8 ng/ml by 6 days. They persist at about these levels for the first 4 weeks of gestation, but there is frequently a transient fall at about 28 days after ovulation to 5 ng/ml (Holtan et al. 1975), followed by a later rise. Published values for progesterone in the blood and plasma vary considerably between laboratories. This is because there are other progesterone-like substances (progestogens) that crossreact with the antisera during the assay; for this reason, several authors refer to this as 'total progestogens'.

In the early part of the second month of pregnancy, the endometrial cups are formed. These are discrete outgrowths of densely packed tissue within the gravid horn, derived as a result of the invasion of fetal trophoblast cells into endometrium, in which they subsequently give rise to the endometrial cup cells (Moor et al. 1975). Usually, there are about 12 cups present at the junction of the gravid horn and body as a circumferential band. The endometrial cups produce pregnant mare serum gonadotrophin (PMSG), which is now referred to as equine chorionic gonadotrophin or equine chorionic gonadotrophin (eCG). It is first demonstrable in the blood 38 to 42 days after ovulation, reaches a maximum at 60 to 65 days, declines thereafter and disappears by 150 days of gestation (Fig. 5.1). The eCG has both follicle-stimulating hormone (FSH)-like and luteinising hormone (LH)-like activity; it is generally assumed that, in association with pituitary gonadotrophins, it

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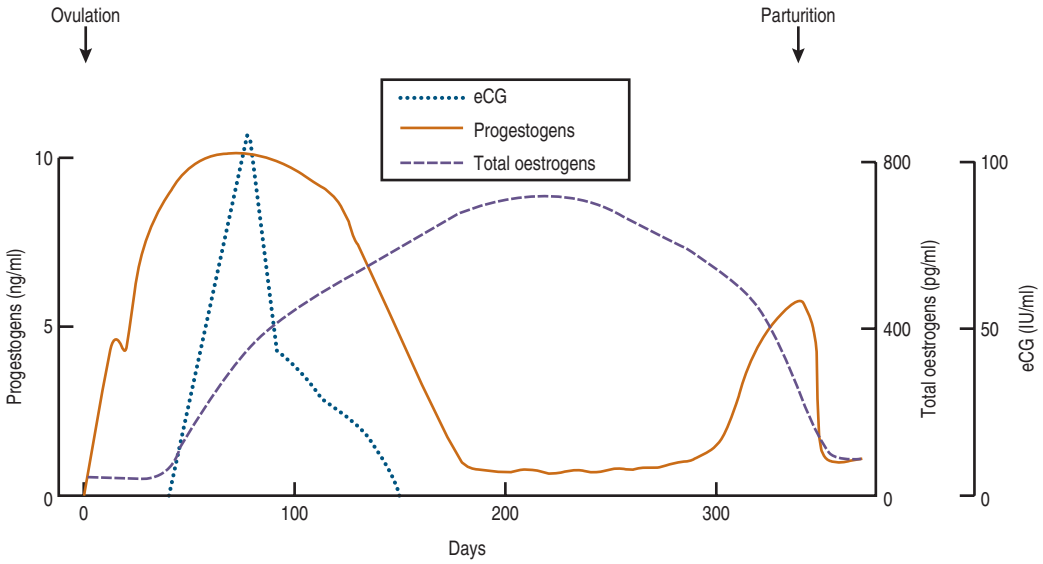

• **Fig. 5.1** Schematic representation of the trends in hormone concentrations in the peripheral circulation of the mare during pregnancy and at parturition. *eCG,* Equine chorionic gonadotrophin.

provides the stimulus for the formation of accessory CLs (Allen 1975) and regulates luteal steroidogenesis (Daels et al. 1998). These structures start to form between 40 and 60 days of gestation, either as a result of ovulation, in the same way that the CL of dioestrus is formed (32%), or as a result of luteinisation of anovulatory follicles (68%) (Squire et al. 1974). Because of the presence of the accessory CLs, the progestogen concentrations in the peripheral circulation increase, to reach and maintain a plateau from about 50 to 140 days and then decline (see Fig. 5.1). By 180 to 200 days the concentrations are below 1 ng/ml; they remain so until about 300 days of gestation, when they increase rapidly to reach a peak just before foaling and subsequently decline rapidly to very low levels immediately after parturition.

Concentrations of total oestrogens in the peripheral circulation during the first 35 days of pregnancy are similar to those of dioestrus, although there is a temporary production of oestrogen by the embryo at 12 to 20 days (Mayer et al. 1977). After this time they increase to reach a plateau between 40 and 60 days at values slightly above those that occur before ovulation, about 3 ng/ml; the rise is probably due to the increased follicular development associated with eCG production. After day 60 it is likely that the increase is due to the activity of the fetus or placenta (Terqui & Palmer 1979). Maximum values are observed at about 210 days, the main source being the fetal gonads (Cox 1975), with a gradual decline towards the time of foaling and a precipitous fall postpartum. The main oestrogens in the mare are oestrone and a ketonic steroid, equilin, as well as oestradiol-17beta, oestradiol-17alpha and equilenin.

Prolactin levels show no distinct pattern, there being considerable variation within and between mares, but there is some evidence of a slight increase towards the end of gestation (Nett et al. 1975).

The main source of progesterone in early pregnancy is the 'true' CL or CL verum (formed from the follicle that ovulated and generated the oocyte that was fertilised) and the accessory CLs. The true CL is active for the first 3 months of gestation and regresses at the same time as the accessory CLs (Squire & Ginther 1975). The placenta must take over the production of progesterone after the regression of the accessory corpora lutea, and, although concentrations fall in the peripheral circulation, they remain high in the placental tissue and must maintain pregnancy by virtue of a localised effect (Short 1957). When ovariectomy is performed at 25 to 45 days of gestation, mares will abort or resorb the fetus; when it is performed after 50 days the response is variable between 140 and 210 days the pregnancy or it continues uninterrupted to term. Thus after 50 days there is evidence of a nonovarian source of progesterone and by 140 days the ovaries are no longer necessary for the maintenance of pregnancy (Holtan et al. 1979).

Changes in the Genital Organs

Conception to 40 Days

Ovaries. The CL *verum* can only be palpated per rectum for 2 to 3 days after its formation. Thereafter, although it persists for 5 or 6 months, it cannot be identified. In pony mares there is some palpable follicular development at about 15 days, whereas during the next 14 days there is quite a marked increase in folliculogenesis with a large number of follicles less than 3 cm diameter, giving the ovaries a 'bunch of grapes' appearance. Ovulations during this period are rare (Allen 1975).

Uterus. During late dioestrus and oestrus, the uterus is soft and the endometrium is oedematous. After ovulation, tone increases and the uterus becomes more tubular. These textural changes are not marked in the nonpregnant animal, in which they subside after the CL begins to regress at 10 to 14 days. However, in the pregnant mare the CL persists, and the tone of the uterus increases to a maximum at 19 to 21 days, when the conceptus causes a soft, thin-walled ventral cornual swelling close to the uterine body. The horn involved is not necessarily on the same side as the ovary which produced the ovum because there is extensive mobility of the conceptus within the horns and uterine body before fixation, which occurs between days 16 and 18. Most clinicians have reported more equine pregnancies in the right horn; for example, Vandeplassche (1957) noted 64%, although in ponies there is only a small majority of right-horn pregnancies. The excess of right-side pregnancies, coupled with the slightly greater incidence of left-side ovulations, indicates a major embryonic migration from left

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to right in horses generally. There is, however, good evidence that other factors can influence the horn in which pregnancy occurs. In a survey of 937 thoroughbred mares, Butterfield and Mathews (1979) found that there was no significant difference between the numbers of right- or left-horn pregnancies: 469 left and 468 right. However, when they examined the results for mares that conceived when they were not lactating, there were a significantly greater number of right-horn pregnancies. There is also good evidence that implantation usually occurs on the opposite side to that of the previous pregnancy. Feo (1980) found that 19 of 22 mares that conceived at the foal heat were pregnant in the opposite horn to that of the previous gestation. Allen and Newcombe (1981) found that in 82.5% of cases the conceptus was present in the opposite horn. Nearly similar percentages were reported by Davies Morel et al. (2009), and these authors also found that the number of pregnancies in the previously gravid horn increased with maternal age and the foaling to conception interval.

The conceptual swelling of the uterine horn protrudes ventrally and craniocaudally but not dorsally and grows slowly during the phase of organogenesis, i.e., until about 30 days. Thereafter, growth is faster, and the swelling progressively extends to the tip of the pregnant horn. Twins are usually disposed at the base of each horn, and in this situation there will be two groups of endometrial cups. If both twins are present in the same horn, only one set of cups will be present.

Vagina and Cervix. During early pregnancy, the vagina becomes progressively paler and dryer and is covered by thin, tacky mucus. The cervix is small and tightly closed and the external os uteri is gradually filled by a plug of mucus and points eccentrically.

40 to 120 Days

Ovaries. This period is characterised by marked ovarian activity, with multiple follicular development causing one or both ovaries to become temporarily larger than during oestrus – in some cases very much larger. Ovulations, forming accessory CLs, and luteinisation of anovular follicles occur. Follicular activity has usually subsided by 100 days, and the CLs begin to regress. In pony mares Allen (1971) found ovulations in pregnancy between 21 and 112 days, with the highest incidences between days 40 to 42, 54 to 56 and 63 to 66.

Uterus. The conceptus completely occupies the pregnant horn by about 60 days, after which the body and then the nonpregnant horn are invaded by the allantochorionic membrane. The pregnant horn now changes from a transverse to a longitudinal disposition in the mare's abdomen. By 100 days the fluid-filled uterus is a somewhat tense swelling on the pelvic brim. At this time the small fetus, closely enveloped in the amnion, floats in a relatively large volume of allantoic fluid.

120 Days to Term

Ovaries. With the gradual regression of all luteal elements and follicles, the ovaries become progressively smaller and harder and are drawn forwards and downwards by the gravid uterus. Except in very large mares, they can usually be palpated throughout pregnancy.

Uterus. Gradual distension of the uterus by the fetus and fluids causes increased tension on the utero–ovarian ligament, and the anterior border of the uterus sinks downwards and forwards. After the eighth month the fetus normally assumes an anterior longitudinal presentation and ventral position (see Chapter 14 for definitions of these terms). Except in very large mares, the fetus can be palpated throughout this period. Fremitus can be detected in the uterine arteries, although it is less obvious than in the cow.

Methods of Pregnancy Diagnosis

Management Methods

Failure to return to oestrus is a good sign that a mare is pregnant. The demonstration of the signs of oestrus (see Chapter 1) usually requires the presence of a teaser stallion, although some mares will respond to geldings and to androgenised geldings. It is preferable that mares should be accustomed to the teasing routine, which should commence 16 days after service and continue for a further 6 days.

False positives will occur for the following reasons:

- The mare has a 'silent heat', a common problem when the foal is at foot.
- The mare becomes anoestrus as a result of lactation or environmental factors.
- The mare has a prolonged luteal phase, yet has not conceived (see Chapter 32).
- The mare has a prolonged luteal phase associated with embryonic death; this is referred to as pseudopregnancy (see Chapter 32).

False negatives will occur in a few mares, which will show oestrus at this time although they are pregnant.

Clinical Methods

Before the availability of ultrasonic methods to detect pregnancy (see later in this chapter), the only clinical methods available were visual inspection of the vagina using a speculum or palpation transrectally using the hand and arm. For experienced clinicians, such methods of pregnancy diagnosis were quick and accurate and, in the absence of the necessary ultrasonographic equipment, still applicable today.

Vaginal Examination

This is best done using a speculum; however, manual exploration can be used. The vaginal mucosa is pale pink, the mucus is scant and sticky, and the cervix small and tightly closed; although the external os is gradually filled with thick, tacky mucus, it is not really apparent as a plug, and points eccentrically.

False positives can occur in early pregnancy because the vagina is indistinguishable from that seen in dioestrus. Errors can also be made as a result of prolonged luteal phase and pseudopregnancy (see Chapter 31).

Rectal Palpation

The presence of follicles during the third week after service does not necessarily indicate that the mare is returning to oestrus. Follicles are normally present during the first 3 months of gestation and give considerable size to the ovaries.

Uterine tone is marked at 17 to 21 days of pregnancy, when the uterine cornua can be palpated as resilient tubular organs. If no conceptual swelling is palpable, then this tone should only be interpreted as suggestive of pregnancy. The uterine body and the nonpregnant horn remain tonic until at least day 50 of gestation. Marked uterine tone may also be found in the puerperal mare mated at the foal heat, when there is acute endometritis and during pseudopregnancy (when early embryonic death is followed by autolysis or expulsion of the conceptus but the uterus retains the texture of pregnancy because of the persistence of the CL).

Palpation of the conceptus is first possible at 17 to 21 days, when it is a small soft swelling of 2.4 to 2.8 cm in diameter or is an apparent 'gap' in the otherwise tonic horn. It is more easily felt between 21 and 30 days, but still only the cranioventral portion of the distension can be appreciated. At 25 days, the conceptual

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swelling is 3 to 3.4 cm. At 30 days, its dorsoventral diameter is 3 to 4 cm. At 35 days, it is 4.5 to 6 cm, and at 40 days it is 6 to 7 cm in diameter, which is about the size of a tennis ball. Thereafter, it is not possible for the conceptual swelling to be completely cupped within the palm of the hand. By 60 days it is becoming oval in shape and measures approximately 13 × 9 cm, whereas by 90 days it has increased to approximately 23 × 14 cm.

There is a natural variation in the size of the conceptual swelling in mares of similar size and ovulation dates because of the variation in the volumes of fetal fluids present. However, swellings that appear small for the given stage of pregnancy should be reexamined later to exclude the possibility of impending resorption (hence the clinician should keep written records of the findings for later comparisons). Twin conceptuses can be identified up to 60 days. After this, a single conceptus is likely to involve both horns and the swelling becomes more diffuse. Care must be taken not to confuse a partially filled urinary bladder with the pregnant uterus during the 70- to 100-day period or with an inflated large colon during days 90 to 120; when in doubt, a search should be made for the ovaries in order to establish an anatomical link between them and the uterus via the utero–ovarian ligament.

At about 100 days it is often possible to ballotte the fetus as it floats in the fetal fluid of the uterine body. Growth of the fetus and reduction in tension of the fetal sacs enable the examiner to palpate parts of the fetus in the uterine body from the end of the fourth month onwards. It may be difficult to locate between the fifth and seventh months in large pluriparous mares and very occasionally in mares near term. The palpable absence of a nonpregnant uterus and tension in the mesovarium are reassuring features.

False-positive results by rectal palpation can be obtained either when, in rare instances, pregnancy is confused with pyometra (see Chapter 32), or when, in the very early stages, the uterine tone due to incomplete involution might be assumed to be due to pregnancy in mares that have been served at the foal heat and in those that have developed a pseudopregnancy. It should also be remembered that mares may suffer embryonic or fetal death with resorption or abortion after they have been confirmed pregnant.

False-negative results can be obtained if there is confusion over the service date, i.e., later than the one recorded, or if the uterus is not palpated completely.

Ultrasonographic Methods

The Doppler and A-mode ultrasonographic methods are of little or no value in the mare; however, B-mode is now commonly used in this species (Fig. 5.2). With this imaging mode and a 5.0 MHz transducer, the earliest gestational age that pregnancy has been confirmed in the mare is 9 days, when the conceptual vesicle appears as a black sphere of about 3 mm in diameter. By 11 days the vesicle has been identified in 98% of ponies and horses examined (Ginther 1986). The conceptus at this stage moves freely within the uterus and has been identified in all parts of the uterine horns and in the uterine body just cranial to the cervix; in fact, it is found more frequently in the body at this stage. Rapid growth of the conceptual vesicle occurs from 9 to 16 days, with evidence of some reduction in growth rate from 16 to 28 days, before it increases. This plateau in growth may be an artefact because, with a reduced turgidity of the vesicle, it is capable of being compressed by the uterine walls. This is also associated with an apparent change in the shape of the vesicle from spherical to oval to triangular, after which it then becomes irregular in outline. Fixation at the base of the horn occurs between 16 and 18 days, when the diameter of the conceptual vesicle is about 19 to 24 mm (Ginther 1986).

Twin ovulations are very common, especially in thoroughbred and draught mares, in which they can occur in up to 30% of ovulations (Davies Morel et al. 2012), although the birth of live twins is relatively uncommon, ranging between 0.8% and 3%, depending upon the breed. The reasons for the discrepancy are:

- Fertilisation failure,
- Death of one or both embryos before or after fixation,
- Death of one fetus, which is relatively uncommon, and
- Abortion of both fetuses; this is the most common sequel and is obviously the most costly one.

Although the detection of double ovulations by B-mode ultrasound has enabled better management and hence prevention of the problem, it is still possible for double ovulations to go undetected. Thus early identification of twin embryos, preferably between days 12 and 14 before fixation occurs, can enable more effective management of the problem (see Chapter 31). For this reason it is important to scan the whole of the uterine body and uterine horns.

In addition to the detection of pregnancy, it is also possible to determine the sex of the foal. However, this requires that the person has good-quality equipment, as well as considerable experience of transrectal ultrasonography and interpretation of ultrasonic images. The method is based on determining the relationship of the genital tubercle to surrounding structures. The genital tubercle is the embryonic precursor of the penis in the male and clitoris in the female. As the fetus develops, the tubercle moves from a position between the hind legs to near the tail in the female, and near the umbilicus in the male. The optimum gestational age is between 59 and 68 days (Curran & Ginther 1989); although accurate results were obtained up to 99 days (Curran 1992), it becomes more difficult after this stage.

Laboratory Methods

Milk or Blood Progestogens. As can be seen in Fig. 5.1, plasma progestogens concentrations in the pregnant mare remain elevated just before or during the time when the mare would have returned to oestrus. Blood or milk samples collected 16 to 22 days after service should have elevated progestogen concentrations in pregnant mares, whereas in nonpregnant mares the levels would be low and typical of those obtained at oestrus. Although Hunt et al. (1978) have quoted 100% accuracy in diagnosing pregnancy using this method, false-positive results occur due to a prolonged luteal phase or activity, which occurs in 20% of mares (Pycock 2007) and is indistinguishable from pregnant values. For this reason the method is not reliable.

Identification of Equine Chorionic Gonadotrophin. Blood samples should be collected, preferably between 50 and 90 days after service, although it is possible to identify the hormone between 40 and 120 days. The test is performed on serum.

Originally, biological methods were used to identify the presence of the gonadotrophin. The most frequently used method was the injection of serum into 3.5-week-old, immature mice, a positive result being the production of ripe follicles in the ovaries and the presence of a swollen enlarged uterus.

Immunological methods are now used – either gel diffusion or the haemagglutination inhibition technique. The latter method is available commercially in simple solid phase ELISA kit with all of the reagents provided, and it is possible for the procedure to be carried out in any veterinary practice laboratory.

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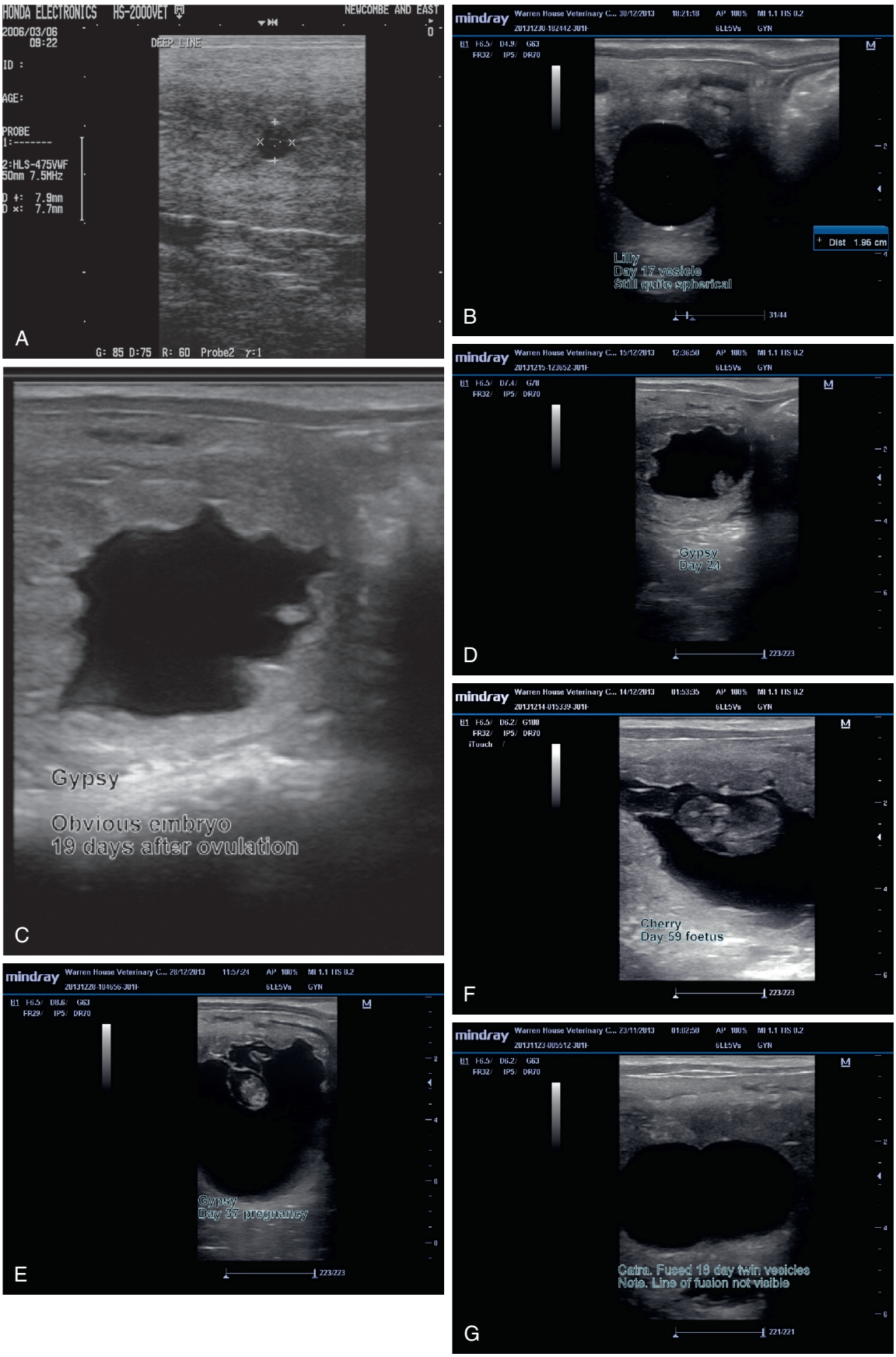

• **Fig. 5.2** Transrectal B-mode ultrasound images of the pregnant uterus of the mare and its contents, using a 7.5 MHz linear transducer. (Courtesy of Dr. J. R. Newcombe.) Images 5.2A to 5.2F are single conceptuses from 13 days after ovulation. Images 5.2G to 5.2K are twin conceptuses. Further brief descriptive details on each image. *Continued*

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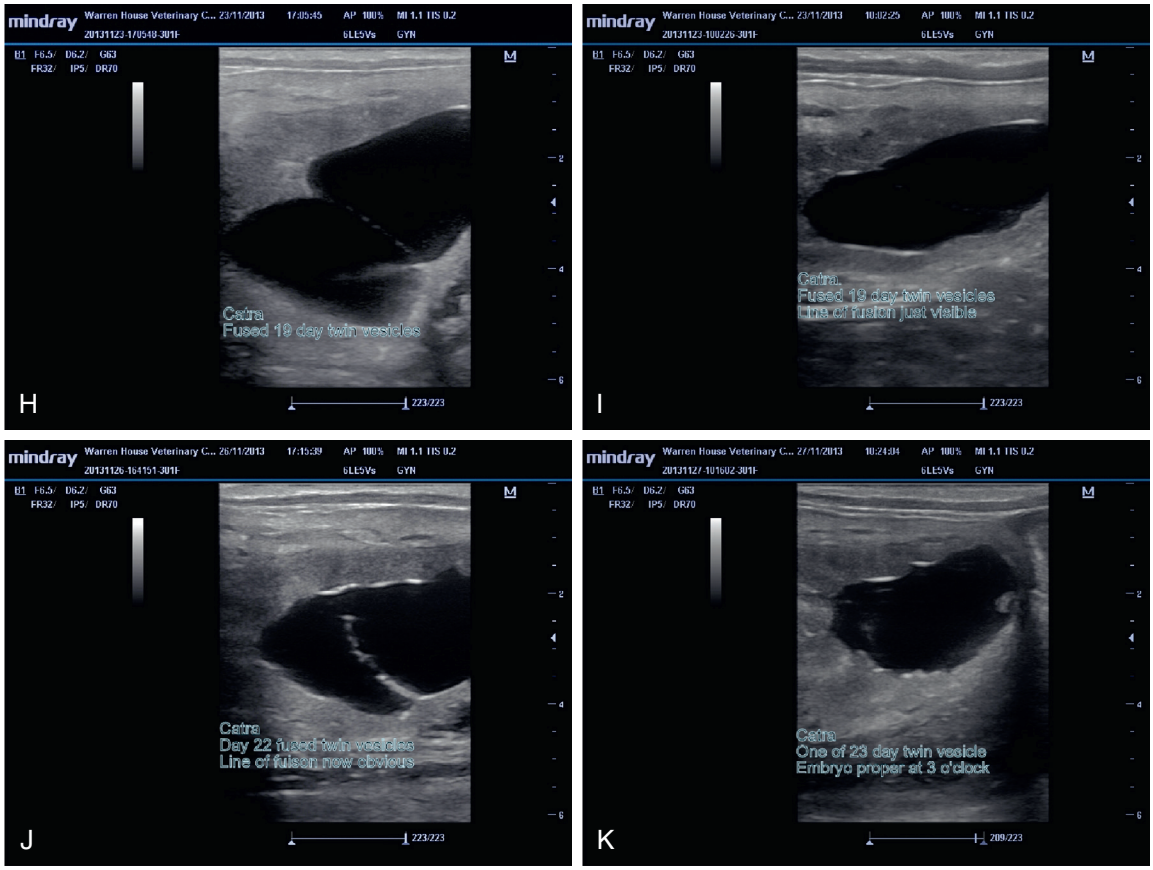

• **Fig. 5.2, cont'd**

False-negative results are obtained if the blood sample is taken either too early or too late, and for this reason it is important to sample at the optimum times stated previously. Some mares produce low levels of eCG that are briefly sustained and cannot be detected using the method.

False-positive results are obtained as a result of embryonic or fetal death, either after the blood sample was collected or in some cases before. Once the endometrial cups have formed, they will persist and still secrete eCG even if the fetus dies. They regress at the time that they would have done if the pregnancy had continued normally. For this reason owners should always be warned of the problems to save subsequent disappointment, and certificates should indicate that a positive result has been obtained with an explanation of the significance of such a result.

Blood Oestrogens. A method of detecting pregnancy by determining the concentration of total oestrogens in the peripheral blood has been suggested by Terqui and Palmer (1979). By 85 days of gestation the concentration should exceed the maximum values obtained in nonpregnant mares.

Urinary Oestrogens. Oestrogens (oestrone and oestradiol-17beta) are present in the urine of pregnant mares in sufficient amounts for accurate detection by a chemical method (Cuboni 1937) between 150 and 300 days of gestation. A modification of this test (Cox & Galina 1970) can be carried out easily.

Because the presence of blood oestrogens depends on a functional placenta, false positives, as seen with the eCG test, do not arise. This chemical test is not sensitive enough to identify the urinary oestrogens of oestrus. It is nearly 100% accurate between 150 and 300 days.

Optimum Time for Diagnosing Pregnancy

The optimum time, as with all species, is as early and as accurately as possible. In thoroughbred mares in particular, in which twin ovulations are common, the early identification of the resultant twin conceptuses, should they be present, is important (see Chapter 31). The use of transrectal B-mode ultrasonic imaging has enabled early identification, which is important because termination of pregnancy in the case of twins must be carried out before the endometrial cups develop. It is important to stress that good results depend upon good equipment and knowledgeable interpretation of the images. Similarly, many of the earlier changes in uterine tone can be identified only by an expert using sequential palpation.

If the mare is not pregnant and has not been observed in oestrus, or if she is in a prolonged luteal phase or anoestrus, the necessary action can be taken (see Chapter 31).

Hazards of Pregnancy Diagnosis

There is no evidence that careful palpation of the genital tract will cause failure of the pregnancy. In those mares in which fetal death occurs after a normal rectal examination has been performed, the pregnancy would have failed in the absence of the examination.

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Pregnancy and Its Detection in Cattle

Endocrinology

The main source of progesterone for the maintenance of pregnancy in the cow is the CL, the placenta producing only small amounts. The results of ovariectomy and removal of the CL are controversial. Up to about 200 days of gestation, removal of the ovary containing the CL, or ablation of the CL, either surgically or with the use of $PGF_{2\alpha}$, usually results in abortion. However, after this stage until just before term, pregnancy very often continues. Placental cells produce progesterone in vitro, and a positive venous/arterial difference of the plasma progesterone concentration was maintained after ovariectomy on day 250 but not on day 270 of gestation (Pimentel et al. 1986). Therefore the placenta may only secrete significant amounts of progesterone during a limited period of gestation (from about days 150 to 265), when it can easily substitute for luteal progesterone after induced luteolysis.

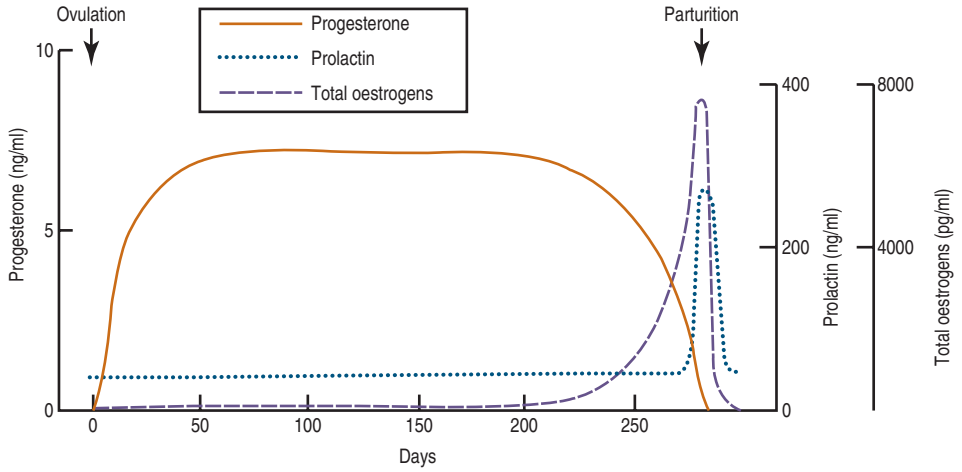
The hormonal changes during pregnancy are illustrated in Fig. 5.3. Progesterone concentrations in the peripheral circulation during the first 14 days of gestation are similar to those of dioestrus; thereafter, those of the nonpregnant cow decline sharply from about the 18th day after ovulation (see Fig 1.29). In the pregnant cow there is normally only a slight decline at this stage, with a rapid recovery. Thereafter, the concentration increases slightly during pregnancy until it starts to decline at about 20 to 30 days prepartum. Oestrogen concentrations during early gestation and midgestation are low, less than 100 pg/ml; however, towards the end of gestation, in particular after day 250, oestrogen concentrations increase to reach peak values 2 to 5 days prepartum of 7 ng/ml estrone sulphate and 1.2 ng/ml estrone (Thatcher et al. 1980). These rapidly decline from about 8 hours prepartum to low levels immediately postpartum.

Both FSH and LH concentrations remain low during gestation and show no significant fluctuations. So there is no evidence to suggest that the prepartum decline of maternal plasma progesterone concentrations, which starts already several weeks before calving, with a more precipitous fall at about 30 to 40 hours before delivery, is due to withdrawal of maternal gonadotrophic support. Prolactin is low during pregnancy until just before calving, when it increases from basal concentrations of 50 to 60 ng/ml to peak values of 320 ng/ml 20 hours prepartum, until a subsequent decline to basal concentrations by 30 hours postpartum. Bovine placental lactogen (bPL) can be detected in the peripheral circulation of the dam after the first month of gestation, increasing dramatically to maximum concentrations between 200 days and term (Bolander et al. 1976, Forsyth 1986). However, other authors reported that maternal levels remain low throughout pregnancy, whereas fetal plasma levels were 7 to 10 times higher at day 90 and gradually declined towards term (Beckers et al. 1982). The physiological role of this placental hormone is still unclear, but it appears to have prolactin and growth hormone-like activities.

Changes in the Genital Organs

Ovaries. In the cow the CL of pregnancy remains at its maximum size throughout the whole of the period of gestation, and a significant decrease is only seen after calving (Ginther 1998). Fundamentally it is indistinguishable from the fully developed CL of dioestrus, but there are certain features by which its persistence can be recognised when examined postmortem. More recently, Scully et al. (2014) performed sequential transrectal ultrasonography of the uterine horns, ovaries and blood flow to and within the ovaries from days 18 to 21 after AI. The best logistic regression model to predict pregnancy included scores for CL blood flow, CL size, and uterine echotexture on day 21 following AI. Accuracy of pregnancy diagnosis was highest on day 21, with sensitivity and specificity being 97.6 and 97.5%, respectively. However, this type of sequential examination would not be practicable in routine veterinary practice.

The chief feature of the CL of pregnancy is that the protrusion of the structure from the surface of the ovary is less marked and the epithelium over it is white and scarred. The CL of dioestrus often contains a central lacuna, whereas during pregnancy this becomes filled. It is considered by some that the CL of pregnancy is larger than that of dioestrus. If this is the case, it is too slight to be of significance; moreover, there are considerable variations in the weights of pregnancy CLs in individuals (3.9–7.5 g), which bear no relationship to the stage of pregnancy. The colour of the CL of pregnancy, however, differs somewhat from that of dioestrus. There is a wider range from yellow through orange to light brown, and the appearance of the luteal tissue is duller. Figs 5.4 to 5.9 show examples of bovine ovaries (natural size) obtained from gravid genital tracts recovered after slaughter. Note the variations in the



• **Fig. 5.3** Schematic representation of the trends in hormone concentrations in the peripheral circulation of a cow during pregnancy and at parturition.

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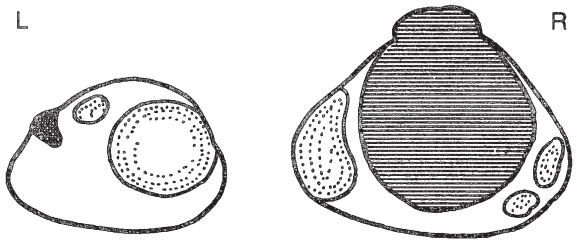
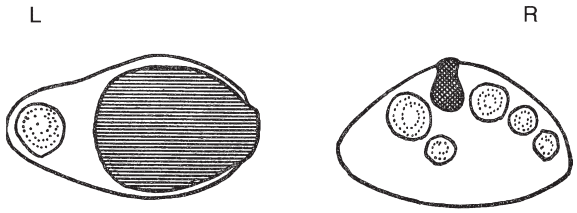
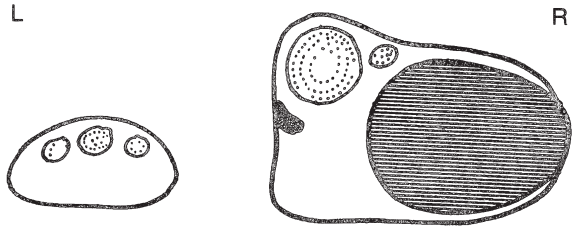


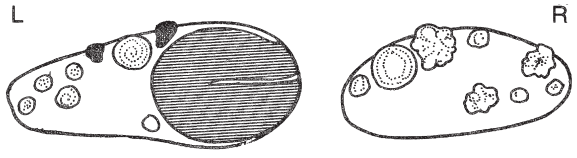
• **Fig. 5.4** Ovaries of the cow at 35 days of pregnancy. Fetal body length 1.6 cm; corpus luteum verum yellow.



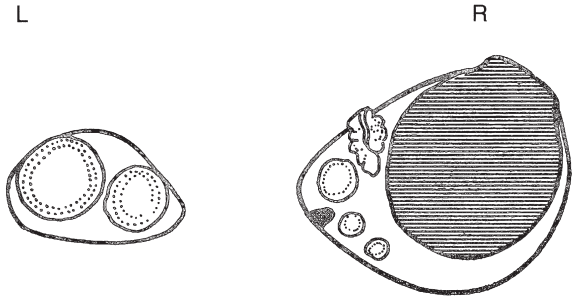
• **Fig. 5.5** Ovaries of the cow at 48 days of pregnancy. Fetal body length 3.4 cm; corpus luteum verum orange.



• **Fig. 5.6** Ovaries of the cow at 70 days of pregnancy. Fetal body length 6.3 cm; corpus luteum verum yellow/orange.



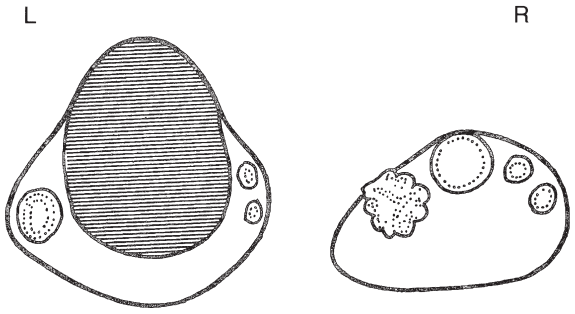
• **Fig. 5.7** Ovaries of the cow at 100 days of pregnancy. Fetal body length 16 cm; corpus luteum verum yellow/brown.



• **Fig. 5.8** Ovaries of the cow at 120 days of pregnancy. Fetal body length 25 cm; corpus luteum verum orange.

shape of the ovaries and the position of the CL of pregnancy. In addition, these sagittal sections also show that folliculogenesis and regression continue throughout pregnancy (Ginther et al. 1996).

As pregnancy advances, the relative position of the ovaries within the pelvic and abdominal cavities changes. Their location, however,



• **Fig. 5.9** Ovaries of the cow at 190 days of pregnancy. Corpus luteum verum orange.

in nongravid animals is not constant. In heifers and young cows they are generally situated on each side of and slightly below the conjoined horns at the level of the pelvic brim. They may lie in the pelvic cavity. In multiparous animals they are often situated in the abdominal cavity 5 to 8 cm in front of the pelvis, where their detection is more difficult. Consequent on the increase in the weight of the uterus and hypertrophy of the ovarian and uterine ligaments, the ovaries pass deeper and deeper into the abdominal cavity as pregnancy advances. From the fifth month onwards the weight and size of the uterus is such that it sinks down into the abdomen to rest on the abdominal floor. Hammond (1927) found the weight of the uterus and its contents in primigravidae at 5 months to be some 48 kg. Provided that the animal is comparatively easy to examine, it is generally possible to palpate the ovaries with reasonable certainty up to day 100, by which time, in heifers, the one on the gravid side is about 8 to 10 cm in front of and slightly below the pelvic brim and that on the nongravid side is a little nearer the pelvis. In occasional cases both ovaries may be detected as late as day 150, although by this time there is a risk that they will be confused with cotyledons. In the later stages of pregnancy it is not so much that they are beyond the reach of the hand as that one is unable to depress the rectum sufficiently deeply into the abdomen to locate them.

Uterus. During the early stages, detection of an increase in size of the uterus affords strong evidence of pregnancy, but the recognition of these changes necessitates an appreciation of the size of the quiescent uterus in subjects of varying ages and parity (Fig. 5.10, Table 5.2), the quantities of fluid present in the respective fetal sacs and the disposition of those sacs in the uterus. Uterine echotexture scores were similar for pregnant and nonpregnant cows from day 18 to 20.

At 28 days of pregnancy the amniotic sac is spherical in outline and about 2 cm in diameter. It occupies the free portion of the gravid horn. The allantoic sac is about 18 cm long, but the amount of contained fluid is insufficient to cause a noticeable (i.e., externally visible) distension and its width is negligible. It occupies almost the whole of the gravid horn. At this stage the embryo is 0.8 cm long, a quite inappreciable size.

At 35 days fetal body length is 1.8 cm, and the diameter of the spherical amniotic sac 3 cm. They still occupy the free part of the horn. The conjoined portions of the horns and the free portion of the nongravid horn are not appreciably changed. It is possible, particularly in a heifer easy of examination, that the distension in the free part of the gravid horn will be detected.

At 60 days the fetal crown–rump length is approximately 6 cm. The amniotic sac is oval and tense, having a transverse measurement of about 5 cm. This causes the free part of the gravid horn to be distended to a width of about 6.5 cm, compared with 2 to

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• **Fig. 5.10** Genital tracts of a nulliparous heifer *(N)* and a pluriparous (seven calves) cow *(P)*.

TABLE 5.2 Dimensions of nongravid genital tract from a nulliparous heifer and pluriparous cow

	Unbred Nulliparous Heifer (cm)	Pluriparous Cow (cm)
Width of conjoined cornua immediately anterior to cervix	2.5	4.0
Width of each cornu at external bifurcation	2.0	3.5
Length of externally connected parts of cornua	9.0	14.0
Length of free portions of cornua	0.5	1.2
Length of cervix	5.0	10.0
Width of cervix	3.0	5.0

3 cm in the quiescent stage in the heifer and young cow. In such subjects this distension may be recognised.

At 80 days the fetus measures 12 cm and the total quantity of fluid is about 1 litre. Distension of the free part of the gravid horn varies from 7 to 10 cm, while that of the conjoined part is but little greater than normal. The greater length of the gravid horn can often be detected.

By 90 days uterine distension is such that it can be detected with accuracy in the great majority of cases. The conjoined horns are tense, the gravid one having a width of about 9 cm and the nongravid one about 4.5 cm. In most individuals the organ is still high up at the pelvic brim; it is generally possible to pass the hand well over the curvature of the distended horn, but in some multigravid cows the uterus lies in the abdomen and to palpate it effectively it is necessary to retract the organ. Sometimes it is possible to detect the fetus at this stage. Tapping the distended horn with the fingers may reveal the fetus rather similar to a piece of wood floating in the fluid beneath. By gently squeezing the uterus one may be able to pick up the fetus; its body length is about 15 cm.

By the fourth month, the uterus sinks below the pelvic brim and distension is less easy to recognise as the fluid gravitates towards the extremities of the horns. The cervix lies on the pelvic brim.

Changes in the size and shape of the gravid uterus during the first 4 months of gestation are shown in Fig. 5.11. A detailed description of the morphology and growth of the bovine conceptus (embryo/fetus, fetal membranes, and placenta) between days 20 and 70 of gestation has been provided by Neto et al. (2010).

Fetus. Several workers (Hammond 1927, Winters et al. 1942) have recorded fetal body lengths (crown–rump) during the various stages of pregnancy. Data have been collected chiefly from pregnant heifers, and thus allowance must be made for the greater size of the fetus in cows, particularly in the later periods of pregnancy (Table 5.3). The fetal bulk in relation to these body lengths will be greater. Changes in several fetal body measures have also been measured repeatedly in the same animals by means of ultrasonography (Kähn 1989, Ginther 1998, Breukelman et al. 2004).

During the period 120 to 160 days it will be possible to palpate the fetus in more than 50% of cases. The presented extremity will lie within reach in front of and below the pelvic brim. In some cases the fetus may be touched transiently at the commencement of examination before it sinks into the depths of the uterus and is beyond reach. Similarly, if a series of examinations of an individual is made during this period, the fetus may be detected on some occasions and not others.

Between 5.5 and 7.5 months the fetus is detected less frequently than during the previous period – perhaps about 50% of the time. In some individuals the fetal head and/or flexed limbs can be palpated just anterior to the pelvic brim. Touching the fetus often provokes reflex movements.

From 7.5 months to the end of gestation the fetus will, in the majority of cases, be palpated readily. However, in some individuals, especially if they are deep bellied, multiparous cows, it may

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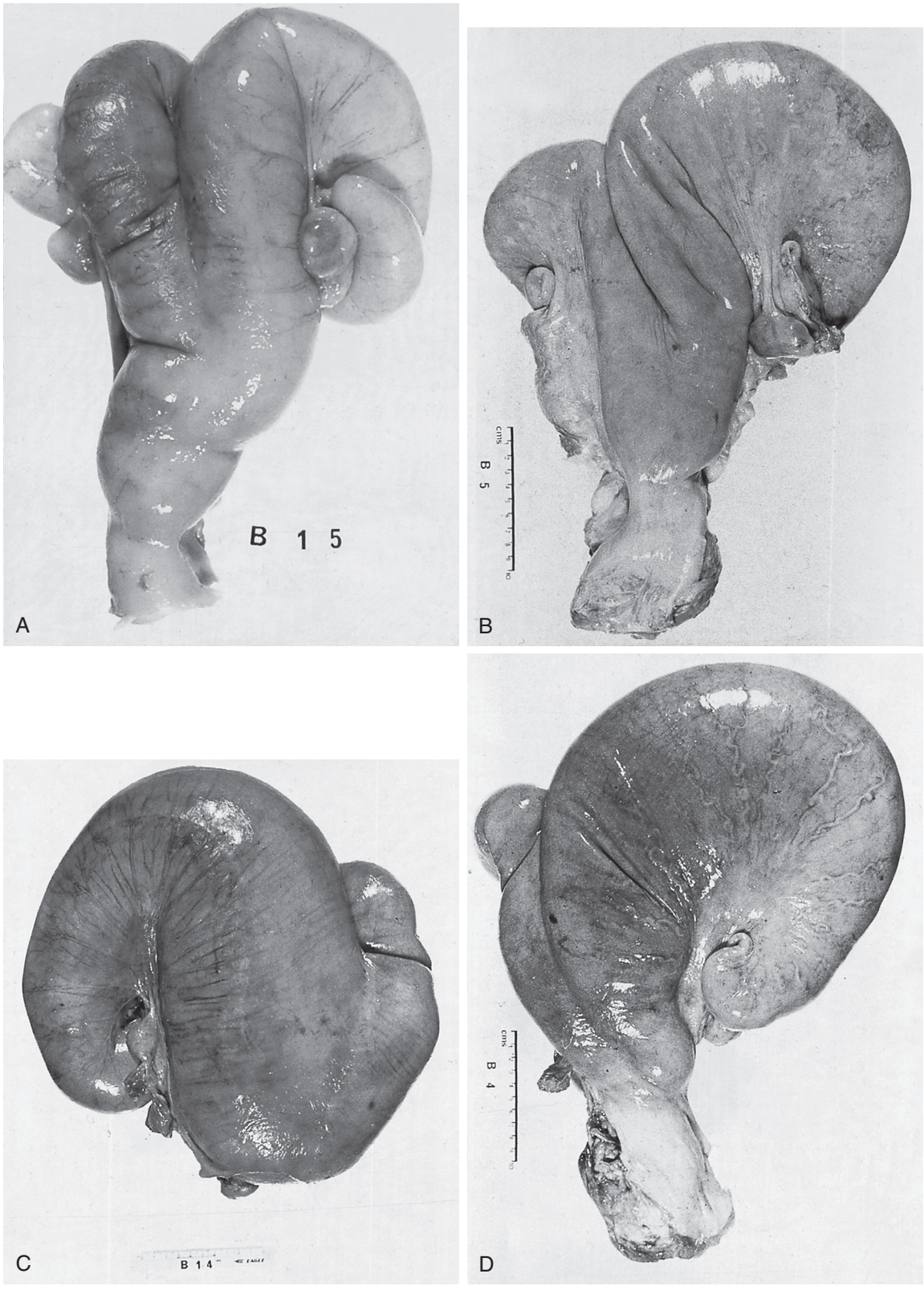

• **Fig. 5.11** Gravid genital tracts from cows at different stages of gestation. (A) Right horn at 6 weeks. (B) Right horn at 12 weeks. (C) Left horn at 16 weeks. (D) Right horn at 19 weeks.

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TABLE 5.3 Fetal body length in cows at various stages of pregnancy

Pregnancy (Months)	Fetal Body Length (cm)
1	0.8
2	6
3	15
4	28
5	40
6	52
7	70
8	80
9	90

not be possible to palpate the fetus on a single examination even at term. Several authors (Ruumlsse 1968, Dufty 1973, Taverne et al. 2002) have shown variations in myometrial tone and activity during late pregnancy. Dufty (1973) found that, in a large number of Hereford heifers examined daily by rectal palpation near term, it was frequently impossible to palpate the fetal calf. The reason for this finding was the considerable relaxation of the myometrium, which allowed the calf to descend into the abdomen.

Nongravid Horn. The extent to which the allantochorionic sac occupies the nongravid horn varies greatly. In the great majority of bovine pregnancies the sac occupies some part of it, in some extending to the apex. In others the caudal two-thirds or one-half only is occupied, whereas in exceptional cases the nongravid horn is entirely unoccupied by fetal membranes. In the majority of cases the nongravid horn also plays its part in placentation and its caruncles hypertrophy, although the degree of caruncular enlargement is not as great as that of the pregnant horn. Occasionally, the nongravid horn, although occupied by the allantochorion, plays no part in placentation, and its caruncles remain undeveloped. In such cases, and also in those in which the nongravid horn is unoccupied, the placentomes in the gravid horn, particularly those in the region of the fetal trunk, become grossly hypertrophied and may at the time of parturition be as large as 8 to 12 cm.

Placentomes. Detection of placentomes is evidence of pregnancy, but variations occur in their size at the various stages of pregnancy in different individuals; this is probably due to differences in numbers, although there is substantial variation throughout the same gravid uterus. Those situated about the middle of the gravid horn are larger than those of the extremities, whereas those in the nongravid horn are smaller than those in the gravid one. Occasionally, there is no placentation in the nongravid horn. As pregnancy continues, they become progressively larger, until in the terminal stages they may be 5 to 6 cm in diameter; however, because the pregnant uterus sinks into the abdomen, it may not be possible to palpate placentomes from the fifth to the seventh month. Development (their number, size, and weight) of placentomes was found to be disturbed in cows carrying fetuses derived from in vitro–produced embryos (Farin et al. 2000, Bertolini & Anderson 2002, Miles et al. 2004).

Uterine Arteries. Evidence of pregnancy is afforded by hypertrophy of the middle uterine arteries and a characteristic change in their pulse wave, which is referred to as fremitus. Blood flow through this enlarged uterine artery has been quantified by means of transrectal Doppler sonography (Bollwein et al. 2002).

Pregnant Side. It is generally accepted that dairy cows are more often pregnant in the right horn and that the CL is in the ovary on the side of the pregnant horn. In a large series of pregnant bovine uteri examined by one of us, the proportion has been 60% right-side pregnancies to 40% left-side. In only one case was the fetus present in the horn opposite to the ovary containing the CL; another case showed a CL of normal size in each ovary with a single fetus in the right horn. In a series of 1506 uteri of dairy cattle examined in the US, Erdheim (1942) found the fetus in the right horn in 1015 (67.4%) and in the left in 474 (31.4%). In a series of 2318 uteri of beef cattle, however, the side incidence of pregnancy was approximately equal: right, 1178 (50.8%) and left, 1121 (48.3%). Among all Erdheim's (1942) specimens there was one exceptional single pregnancy in which he found the CL in the left ovary and fetus in the right horn. Of 133 pregnant uteri from Swedish Highland cattle, Settergren and Galloway (1965) found 59.4% pregnant on the right side and 40.6% on the left. This series also included one specimen in which the CL was in the left ovary and the fetus in the right horn. So transuterine migration of the embryo before implantation appears to be really exceptional in cows. Unequal distribution of sexes within the left and right uterine horns has recently been reported: the sex ratio (% males) of fetuses in the left horn was significantly lower than the sex ratio in the right horn (Giraldo et al. 2010). The mechanism underlying this disparity remains to be elucidated.

Twinning. In the past, the incidence of twinning was about 1% in dairy cattle and 0.5% in beef cattle. Individual breed records showed higher figures: 2.7% to 8.85% for Brown Swiss; 3.08% to 3.3% for Holsteins; 2.8% for Ayrshires; 1.95% for Guernseys; and 13% for Jerseys (Meadows & Lush 1957, Johansson 1968). The rate increases with age, with figures for Holsteins showing 1.3% for heifers rising to 7% in 10-year-old cows. In dairy cattle, twinning rates have significantly increased over the past decades, alongside the increase in milk production (Nielen et al. 1989), with double ovulations reaching more than 20% and twin birth rates exceeding 9% in very high-producing animals. In an 11-year study involving 12,589 calvings in Friesian-Holsteins, the average twinning rate was 5% to 6%, with yearly values ranging from 3.9% to 7.7% (Andreu-Vazquez et al. 2012).

In the majority of cases, one CL is present in each ovary and a fetus in each horn. Not infrequently, however, two CLs are found in a single ovary but with a pregnancy in each horn. In a study of 211 cases within a large dairy farm (Lopez-Gatius & Hunter 2005), 41% were found to be bilateral and 59% unilateral twin pregnancies, with at least two CLs present in each of the animals. This observed natural preponderance of unilaterally disposed twins does not conform to the experimental results of Rowson et al. (1971), which showed that induced twin pregnancies were more stable when an embryo was transplanted into each horn than when two embryos were placed in one horn. Arthur (1956) and Erdheim (1942) each encountered only one case of identical twinning in which there was a single CL associated with two developing fetuses. The aggregate recorded statistics for identical twinning is 4% to 6% of all twins. The incidence of bovine triplets is about 1 in 7500 single births.

Methods of Pregnancy Diagnosis

A variety of different methods have been used to detect the presence or absence of pregnancy in the cow. These range from the

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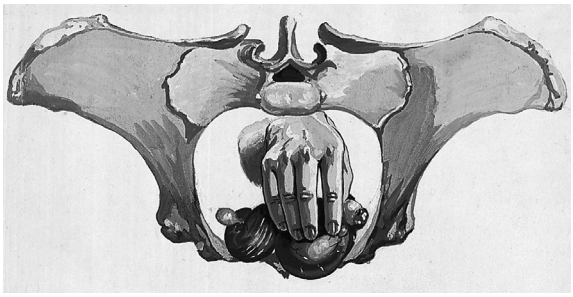
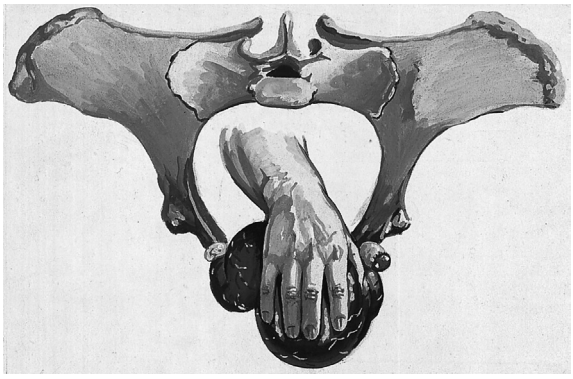
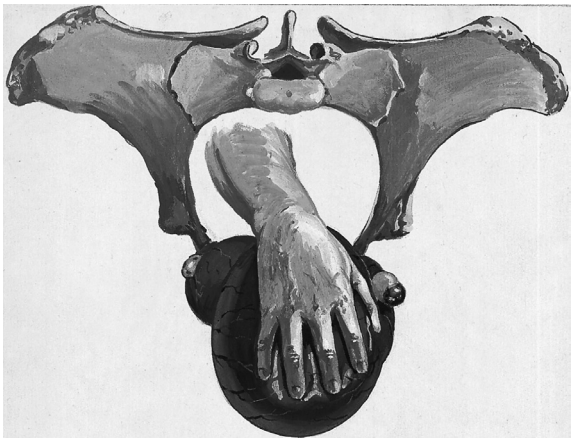


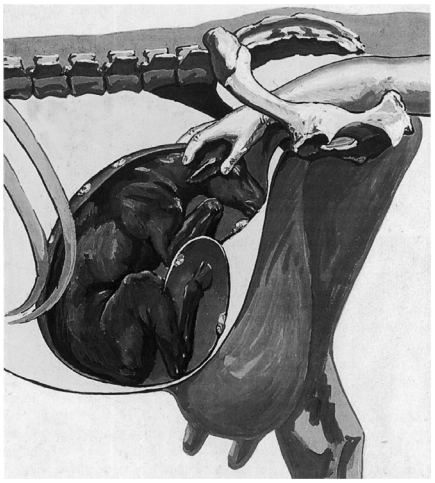
• **Fig. 5.12** Detection of pregnancy in the cow by transrectal palpation. Uterus gravid 70 days.



• **Fig. 5.13** Detection of pregnancy in the cow by transrectal palpation. Uterus gravid 90 days.



• **Fig. 5.14** Detection of pregnancy in the cow by transrectal palpation. Uterus gravid 110 days.



• **Fig. 5.15** Detection of pregnancy in the cow by transrectal palpation. Pregnancy approaching term.

TABLE 5.4 Methods of pregnancy diagnosis in cattle and the times in gestation when they can be first used

Diagnostic method	Day of Gestation
Real-time ultrasound (direct imaging)	13–21
Failure to return to oestrus and persistence of CL	21
Progesterone concentration in plasma or milk	21–24
Assay of pregnancy specific protein B (PSPB) or pregnancy-associated glycoprotein (bPAG)	24
Palpation of the allantochorion ('membrane slip')	33
Unilateral enlargement of horn and disparity in size, thinning of the uterine wall, fluid-filled fluctuation of enlarged horns	35
Palpation of the early fetus when amnion loses its turgidity	45–60
Palpation of placentomes	80
Hypertrophy of the middle uterine artery until presence of fremitus	85
Oestrone sulphate in blood and milk	105
Palpation of fetus	120

identification of substances that are present in body fluids using laboratory assays and from different ultrasound modes to simple clinical methods such as transrectal palpation; the latter has been the most ubiquitously used method for the last 70 years (Figs 5.12–5.15). Table 5.4 lists the methods of pregnancy diagnosis and the earliest times that they can be used. In terms of efficient livestock production, it is the early identification of the nonpregnant cow that is important because immediate measures can be taken to attempt to induce pregnancy again as soon as possible.

Management Methods

Failure to Return to Oestrus and Persistence of the CL. Failure of regression of the CL at about 21 days, as determined by transrectal palpation or ultrasonography, provides a method of anticipating that the cow is probably pregnant. It is seldom used as a practical procedure, and there are reasons for the CL persisting in the absence of pregnancy (see Chapters 22 and 23). Rectal examination at about this time in an individual that was close to or in oestrus would demonstrate the presence of a turgid, coiled uterus and a mucoid vaginal discharge.

How reliable is failure to return to oestrus as a method of diagnosing pregnancy? This depends on the efficiency and accuracy of oestrus detection. In a large group of dairy cows it can be expected that about 50%, or fewer in many cases, will become pregnant after artificial insemination and thus 50% will not. Those that are pregnant will not return to oestrus, whereas those that are

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not pregnant will return. However, a substantial number returning to oestrus will not be detected (see Chapter 22) and thus will incorrectly be assumed to be pregnant, although there will be an opportunity for detection at subsequent oestruses. Assuming an oestrus detection rate of 60% (see Chapter 22), 30% of cows will be incorrectly assumed to be in calf. This constitutes a large error and fully justifies the use of other diagnostic procedures. In addition, following early pregnancy diagnosis, there is always a chance that the pregnancy will fail, and if the cow is not seen to return to oestrus, she will incorrectly be assumed to be pregnant.

Mammary Glands. Mammary changes during pregnancy are best observed in primigravida. The teats of the pregnant heifer begin to enlarge about the fourth month, and, with a little experience, it is an easy matter to distinguish them from those of the nonpregnant or early pregnant animal. From the sixth month the mammary glands become more firm to the touch, and their enlargement can be seen. Hypertrophy is progressive and is particularly marked during the terminal month. As parturition approaches, the glands become grossly enlarged and oedematous, and the teats take on a waxy, tumefied appearance. The abdominal wall, particularly in the region of the umbilicus, may also become swollen by oedema. In the dry milch cow, mammary enlargement occurs during the last 14 or so days of pregnancy. After the fourth month a honey-like secretion may be withdrawn from the teats of pregnant heifers.

Abdominal Ballottement. This is often possible as early as 7 months of gestation in some small breeds such as the Jersey. However, in fat cows of large breeds it is sometimes impossible even at term. The method involves fairly vigorous pummelling of the ventral abdomen and flank with clenched fists. The object is to push the fetus, which is floating in the fetal fluids, away from the body wall and then identify it as it swings back against the fist, which is kept pressed against the abdominal wall.

Laboratory Methods

Identification of Early Pregnancy Factor/Early Conception Factor. Early pregnancy factor (EPF) is an immunosuppressive glycoprotein associated with pregnancy. It was first identified in the mouse (Morton et al. 1974) and subsequently in a large number of domestic species; in the cow it has a molecular weight of 200,000 (Threlfall 1994). Commercially available test kits have been available to detect early conception factor (ECF) in serum and milk from as early as 3 days after artificial insemination, although more accurate results are obtained if samples are taken later, at 7 to 8 days (Adams & Jardon 1999, Threlfall & Bilderbeck 1999). However, in a more recent report the accuracy of a commercial test still appeared to be unacceptably low (Cordoba et al. 2001). Obviously an accurate early pregnancy test would have important practical application in the early identification of the nonpregnant cow in the luteal phase after an unsuccessful insemination; thus the cow can be treated with $PGF_{2\alpha}$ to induce a premature oestrus, when it can be rebred, thus saving time (Chapters 8 and 22). It may be that, in time, such a test will be available.

Assay of Bovine Pregnancy-Specific Proteins B or Bovine Pregnancy-Associated Glycoproteins. These bovine pregnancy-specific proteins B (bPSP-Bs), also known as bovine pregnancy-associated glycoproteins (bPAGs), are members of a large family of glycoproteins that belong to the subclass of aspartic proteinases. Their biochemical and structural characteristics have been extensively reviewed by Sousa et al. (2001). They have been identified in the maternal serum and milk of cows from 24 days of gestation onwards; their concentrations are measured by radioimmunoassays (Sasser & Ruder 1987, Zoli et al. 1992a). Plasma levels rise progressively between the 6th and 35th week of gestation, but they increase steeply during the final 6 weeks and reach very high levels (on average 2460 ng/ml) a few days before calving (Zoli et al. 1992a). The proteins, present in many different glycosylated isoforms, are secreted by the binucleate cells of the trophoblastic ectoderm (Reimers et al. 1985, Zoli et al. 1992b), and thus their presence can be used to confirm pregnancy. However, because they have a long biological half-life (some 3 days) and plasma levels are extremely high around calving, the proteins can also be identified in serum for up to 7 weeks postpartum; for the same reason false positives (i.e., noncalving cows tested as pregnant) can occur after spontaneous (Szenci et al. 2000, Chavatte-Palmer et al. 2006) or induced embryonic or fetal death (Szenci et al. 2003, Breukelman et al. 2005a).

PSPBs or bPAGs were initially mainly measured by autologous or heterologous radioimmunoassay, but development and testing of suitable enzyme-linked immunosorbent assay (ELISA) methods have been developed, enabling the use of an on-farm diagnostic test (Sasser & Ruder 1987). Using radioimmunoassay to detect PSPB, Humblot et al. (1988) reported 90% accuracy at 30 days of gestation. It has been shown that there is a good correlation between PSPB in peripheral plasma concentrations and fetal numbers, and thus the method could be used to identify twins (Dobson et al. 1993, Patel et al. 1995). However, such a test does not appear to be sufficiently accurate before day 85 (Szelenyi et al. 2015). In a field study (Szenci et al. 1998), the accuracies of both a bPAG and PSPB radioimmunoassays method were compared with that of transrectal ultrasonography (Table 5.5).

Karen et al. (2015) performed a study in which 100 cows were examined 28 days after AI, and 41 were confirmed pregnant, based on the actual gold standard for pregnancy diagnosis: Transrectal ultrasonography (TRUS) at Day 42. The sensitivity of TRUS, a commercial PAG-ELISA test and a PAG-RIA test for diagnosing pregnant cows at Day 28 were 92.7%, 90.2% and 100%,

TABLE 5.5 Comparison of the accuracies of three different methods of early pregnancy diagnosis, applied in the same cows at three different stages after artificial insemination

	ULTRASONOGRAPHY			BPAG-1			BPSP-B		
Days After Artificial Insemination	29/30	33/34	37/38	29/30	33/34	37/38	29/30	33/34	37/38
Sensitivity (%)	90	97	100	95	95	96	92	98	100
Specificity (%)	96	99	99	69	77	72	83	87	84

Detection of allantoic fluid during transrectal scanning with a 7.5 MHz linear array transducer was used as a positive criterion in the ultrasound test. In the bPAG-1 radioimmunoassay a value > 0.8 ng/ml and in the bPSP-B assay a value > 0.5 ng/ml was used as a positive criterion.
(After Szenci et al. 1998)

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respectively, whereas the specificity of the three tests for diagnosing nonpregnant cows were 91.5%, 98.3% and 94.4%, respectively. The degree of agreement (Kappa±S.E.) between PAG-RIA and PAG-ELISA test was 0.90 ±0.04. The degrees of agreement between PAG-RIA and PAG-ELISA and TRUS at Day 28 were 0.80±0.05 and 0.76±0.06, respectively. Thus the commercial PAG-ELISA test is a highly accurate method for diagnosing early pregnancy in dairy cows on Day 28 after AI, and may be used as an alternative method to the TRUS and the PAG-RIA test.

Recently, circulating nucleic acids (CNAs) have been used successfully as biomarkers for the detection of early pregnancy in cattle (Mayer et al. 2013).

Progesterone Concentration in Plasma and Milk. In 1971 Robertson and Sarda described a method of diagnosing pregnancy by the determination of the progesterone concentration in the plasma of cows. Because the CL persists as a result of the pregnancy, if a blood sample is taken at about 21 days after the previous oestrus, progesterone levels will remain elevated. If the cow is not pregnant and is close to or at oestrus, then the progesterone levels will be low; this can be seen if the progesterone curves in Figs 1.29 and 5.3 are studied. Although this is a perfectly valid and reliable laboratory method, it has the one disadvantage in that it requires the collection of a blood sample.

In 1969 Heap et al. showed that progesterone crossed the mammary gland and appeared in milk. Laing and Heap (1971) confirmed that the changes in progesterone concentrations in the milk closely followed those in the blood or plasma. Furthermore, because progesterone is very soluble in milk fat, there were higher concentrations per unit volume in milk than in the blood or plasma. Heap et al. (1973) described the use of the technique to diagnose pregnancy, and since then a large number of different workers in many different countries have described similar methods. Initially, the technique depended upon the herdsperson collecting about 20 ml of milk, usually at the afternoon milking because the fat content is higher, into a glass or plastic bottle. Then a tablet of potassium dichromate and mercuric chloride as a preservative is added, and provided that the sample is not exposed to high temperature or excessive ultraviolet light, there is very little loss of progesterone activity. At present, an 'in-line' milk progesterone assay is not available, although one system, the 'Delaval Herd Navigator Advanced Analysis System', can measure P4 values in milk; however, this is done after removal of milk from the 'milk line' for analysis by an adjoining instrument (Dobson 2016). So far an 'in line' method has not been developed, but it is likely that one will be designed fairly soon.

Initially, progesterone concentrations in the milk were assayed using radioimmunoassay, which is an effective method of measurement but requires the use of radioisotopes and the equipment to measure radioactive emissions. It can only be performed in a specialist laboratory and hence has the big disadvantage of taking several days before a result is known.

A number of qualitative cow-side tests have been developed that can be used on the farm and hence enable the herdsperson to obtain a result within 1 hour of collecting the milk sample. All the necessary reagents and equipment are provided in kit form. Semiquantitative or fully quantitative tests are also available, but these are designed for use in a veterinary practice laboratory because they require a minimum amount of equipment and some expertise. Both tests are based on ELISA.

The laboratory-based quantitative tests have a series of progesterone standards that enable a standard curve to be drawn. It is usually recommended that duplicate assays are done for each sample, at least until the operator becomes conversant with the procedure. There are a few problems in using the assay on the farm. These can be summarised as follows:

- Instructions are not always readily understood by persons not used to laboratory procedures.
- Simple equipment requires a fair degree of manual dexterity.
- Instructions should be closely adhered to, particularly with regard to timing of incubation and quantities of reagents.
- The kits should be kept in a refrigerator at 4°C and should be allowed to warm to room temperature before use; they should not be heated.
- Interpretation of the colour differences can be difficult for some persons.
- Milk samples should be kept at 2°C to 8°C until assayed, and the recommended preservative tablets must be used.

The optimum time for collecting the milk sample is 24 days after service or artificial insemination (Heap et al. 1976). This time interval prevents those cows with a longer-than-average interoestrous interval from giving false-positive results; however, in those animals with a shorter-than- average interoestrous interval, false positives will occur. The accuracy of the method in the diagnosis of pregnancy is between 80% and 88% (Heap et al. 1976, Hoffmann et al. 1976, Koegood-Johnsen and Christiansen 1977); the accuracy of the method for detecting the absence of pregnancy is nearly 100%. However, Pieterse et al. (1990) reported quite different values from a field study in which milk samples were taken on the day of insemination and on day 21: sensitivity was 86% and 93% for a radioimmunoassay and a cow-side enzyme immunoassay test respectively, whereas specificity was only 48% (radioimmunoassay) and 39% (enzyme immunoassay). Recently, a milk progesterone lateral flow test (LFT) has been described (Waldmann and Raud 2016). It is an immunochromatographic method that utilises an immunostrip for nonlaboratory diagnostic purposes; thus it is a true 'on-the-farm' method. The level of accuracy of the LFT compared with the EIA was 95% for milk samples containing less than 2 ng/ml progesterone and 97% for milk samples containing more than 10 ng/ml progesterone. The validation of oestrus by the LFT was 98.6% accurate using 2 ng/ml progesterone as the EIA estimate for oestrus. The test performance for a nonpregnancy diagnosis was subject to the day of milk sampling, showing the highest accuracy on day 24 post insemination for both tests. When optimised for maximum specificity and compared with rectal palpation, the LFT had a sensitivity and specificity for nonpregnancy diagnosis on day 24 post insemination of 75% and 100%, respectively, with an overall accuracy of 84.4%. The corresponding characteristics for the quantitative EIA were 85%, 100% and 90.6%, respectively. The LFT results compared favourably with the quantitative milk progesterone EIA.

The reasons for false-negative results are:

- Mistaken identity of the animal either on the farm or in the laboratory,
- Milk storage problems due to excessive heat or ultraviolet light,
- Low progesterone production by the CL, and
- Inadequate mixing of milk so that a low-fat sample is obtained.

The reasons for false-positive results are:

- Cows with shorter-than-average interoestrous intervals, i.e., 18 days: When milk samples are taken 24 days after service or artificial insemination, if the cow is not pregnant she will already be in the early luteal phase of the next cycle;
- Embryonic death, if it occurs after the day when the milk was collected (see Chapter 22);
- Luteal cysts that produce progesterone (see Chapter 22);
- Incorrect timing of insemination: Several reports (Hoffmann et al. 1976) have shown that up to 15% of cows are presented

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for artificial insemination when they are not in oestrus. Thus if a milk sample is taken 24 days after the cow was incorrectly inseminated in early dioestrus or middioestrus and the intervening oestrus has not been observed, then she will be in the subsequent dioestrus with a functional CL and elevated milk progesterone concentrations; and

- Pathological prolongation of the life span of the CL; this will be discussed in Chapters 22 and 23.

The main advantage of the milk progesterone test is that it identifies those cows that are not pregnant before it is possible to do so by some other methods such as transrectal palpation. A 24-day sample will then enable the herdsperson to anticipate the return to oestrus 42 days after the service if the cow is not pregnant or enable the veterinarian to examine the animal if she is a problem before she returns again. Cows that are found to be pregnant at 24 days should be examined at a later date by transrectal ultrasonography or palpation. The on-farm tests can be used as early as 19 days after service because a low progesterone concentration at this time is indicative of nonpregnancy, and thus the first return to oestrus can be anticipated. Daily sequential samples can be taken at or around this time, but it is expensive and time-consuming.

Isobe et al. (2005) have demonstrated that faeces collected between days 18 to 24 after artificial insemination or days 11 to 17 after embryo transfer can be used to measure progesterone for the purpose of pregnancy diagnosis in dairy and beef cattle.

Oestrone Sulphate in Milk. Oestrone sulphate is quantitatively one of the major oestrogens in the milk of pregnant, lactating cows (Hatzidakis et al. 1993). During gestation the concentration increases gradually, so that after day 105 it is present in the milk of all pregnant animals, whereas in nonpregnant individuals it is low or undetectable; the source of the hormone is the placenta. The identification of oestrone sulphate in the milk of a cow at 105 days of gestation or later is a very reliable method of pregnancy diagnosis (Hamon et al. 1981). Furthermore, unlike progesterone assays, the precise date of sampling is not required. However, it has limited applications because of the lateness of the time that a positive diagnosis can be made.

Clinical Methods

Transrectal Palpation

Despite the increasing availability of ultrasonic methods (see later in this chapter), traditional palpation will always be important as part of the normal clinical examination of the reproductive tract in cows.

Palpation of the Amniotic Vesicle. This method involves the palpation of the amnion towards the end of the first month of pregnancy. It proceeds briefly as follows: The bifurcation of the uterine horns is located; then the horns are uncoiled and gently palpated along their entire length between the thumb and middle two fingers. The amniotic sac can be felt as a distinct, round, turgid object, 1 to 2 cm in diameter, floating in the allantoic fluid. The vesicle should not be compressed directly but gently pushed backwards and forwards. Some (Rowson & Dott 1963, Ball & Carroll 1963, Zemjanis 1971) consider that this technique is dangerous because of the possibility of rupture of the amniotic sac or of the embryonic heart. As with all rectal techniques, care is the rule, and excessive pressure and rough handling should be avoided.

Palpation of the Allantochorion (Membrane Slip). This method is dependent upon the facts that, in the cow, attachment of the allantochorion to the endometrium occurs only between the cotyledons and the caruncles and that the intercotyledonary part of the fetal membrane is free. The method was first described by Abelein in 1928 (see Cowie 1948), who reported that it could be used from the fifth week of gestation. The method is as follows: Identify the bifurcation of the uterine horns, pick up the enlarged gravid horn between thumb and either index or middle finger, just cranial to the bifurcation and gently squeeze and roll the whole thickness of the horn. The allantochorion will eventually be identified as a very fine structure as it slips between the thumb and finger, before the uterine and rectal walls are lost from the grasp. It is important in the early stages of pregnancy to grasp the whole width of the horn because as the allantochorion is very thin at this stage the structure that can be more readily identified is the connective tissue band that contains the blood vessels supplying the allantochorion (see Figs. 4.5, 5.11, and 5.12). Fincher (1943) recommended that this method should not be used before 40 days of gestation and that it was infallible up to 95 days. The advantage of the method is that it enables the differential diagnosis of pregnancy from mucometra or pyometra. In some cases, particularly after 60 days of gestation, it can be more readily elicited in the nongravid horn because the tension on the wall is less, and this allows it to be grasped more readily. For the beginner it is a worthwhile exercise to practice it on a fresh gravid genital tract from the abattoir.

Unilateral Horn Enlargement. Unless there are twin conceptuses, one in each horn, it is possible to detect a difference in the size of the two horns. This is largely due to the presence of fetal fluids, in particular allantoic fluid, which gives the uterine horn a fluctuating feel with good tone. It can be likened to the feel of a balloon that has been filled with water to a point when the wall just starts to stretch. At the same time, if the wall of the horn is squeezed, it is noticeable that it is much thinner than that of a nongravid tract.

In many cases, a definite diagnosis of pregnancy can be made on these signs alone. The presence of a CL in the ovary adjacent to the enlarged horn is a useful confirmatory sign although a false diagnosis of pregnancy may be made in cases of pyometra, mucometra or incomplete uterine involution (see Chapter 7).

Palpation of the Early Fetus. At about 45 to 50 days of gestation, the amniotic sac becomes less turgid, and it is sometimes possible to palpate directly the small developing fetus. This should be done with care.

Palpation of Placentomes. Placentomes first become recognisable by rectal palpation at 10 to 11 weeks as roughened elevations when the fingers are passed back and forth over the surface of the enlarged gravid horn. From about 3 months they can be identified as discrete structures in the midline, about 8 to 10 cm in front of and over the pelvic brim, by pressing down upon the uterine body and base of the horns. In the early stages it is difficult to identify them as distinct, individual structures. The uterus feels as if it has an irregular corrugated surface; it has been likened to palpating a 'sackful of small potatoes'. As pregnancy proceeds, the placentomes become larger, but once the uterus has sunk into the abdomen between 5 and 7 months, it is sometimes impossible to palpate them. Substantial pressure should be placed on the body of the uterus just cranial to the cervix with the flat portion of the fingertips, as if attempting to palpate the udder transrectally. Identification of placentomes is virtually diagnostic of pregnancy, but in the immediate postpartum uterus, they can also be felt.

Palpation of the Cervix. Evidence of pregnancy can be assumed when there is tension on the cervix. In the nonpregnant or early pregnant cow or heifer, the cervix is freely movable from side to side. However, as pregnancy advances the cervix becomes less mobile and is pulled forwards and downwards over the pelvic brim.

Hypertrophy of the Middle Uterine Artery and Development of Fremitus. In a nongravid or early pregnant cow, identification of the middle uterine artery by palpation is usually not possible

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transrectally. The artery runs in the broad ligament, along a tortuous course, passing downwards, forwards and towards the midline over the pelvic brim close to the junction between pubis and ilium. Usually, it is identified 5 to 10 cm lateral to the cervix. Inexperienced persons sometimes confuse it with the iliac and obturator arteries, but the middle uterine artery is mobile and can be encircled within the thumb and forefinger. At some stage during pregnancy it will cease to have the usual pulse, and instead, this will become a thrill or tremor, which is called fremitus.

There is considerable variation in the time at which the change can first be felt and also when it becomes continuous. The earliest one of the authors (D.N.) has been able to detect it is 86 days. During the period 100 to 175 days, cases will frequently be met in which the initial fremitus becomes a conventional pulse. It is probable that the degree of pressure applied to the artery influences the feeling imparted to the fingers – light pressure detects fremitus whereas a pulse wave is apparent to heavy pressure. Fremitus generally becomes continuous after day 175, although cases will be met in which there is distinct pulsation as late as day 200. During the terminal stages of gestation, the uterine arteries become greatly hypertrophied and tortuous and can be distinctly felt about 8 to 10 mm in diameter with a continuous, tremor-like pulse, laterally situated 2 cm or so in front of the cranial border of the iliac shaft. A difference in size of the two uterine arteries is usually recognisable from about day 100, and this indicates the side of the pregnant horn. Palpation of the middle uterine artery is usually possible in even the biggest cow and thus is particularly useful in large suckler cows.

Palpation of the Late Fetus. Palpation of the fetus, either per rectum or by abdominal ballottement, is diagnostic of pregnancy. The ease of palpation depends upon the size of the cow, the degree of stretching of the suspension of the uterus and the degree of relaxation of the rectum and uterine wall.

Accuracy of Pregnancy Diagnosis by Transrectal Palpation. The most likely reason for an apparent false-positive diagnosis is that there is subsequent embryonic or fetal death. Other reasons for false positives are: incomplete uterine involution (see Chapter 7), pyometra, mucometra, and hydrometra (see Chapters 23 and 24) and failure to retract the uterus. The reasons for false negatives are incorrect recording of the date of service or artificial insemination, so that when the cow is examined she is pregnant but a cycle length earlier than expected, and incomplete retraction of the uterus. There are two reasons for false negatives. Firstly an error in the recording of the date of service or artificial insemination, so that when the cow is examined she is pregnant, but a cycle length earlier than expected, secondly incomplete retraction of the uterus. The latter reason is worthy of further consideration and can be a particular problem in large pluriparous cows with deep abdomens. In order to make a complete examination of the uterus by palpation, full retraction is necessary. The inexperienced person may well make a diagnosis of pregnancy because the uterus is 'out of reach' and cannot be palpated. It is important that the diagnosis should be made on the identification of positive signs. It is perfectly permissible to admit uncertainty, to note in writing the changes that can be identified and to reexamine the animal 2 to 3 weeks later.

Induced Prenatal Death Due to Transrectal Palpation. Concern is sometimes expressed that rectal palpation can induce embryonic or fetal death. There have been several studies to evaluate the risks, by recording if a cow failed to calve having previously been diagnosed as pregnant either by rectal palpation or, more recently, in association with milk progesterone assays or ultrasonography. The results have been equivocal, but although it is possible that certain methods and certain individuals may increase the incidence of prenatal death, it is likely that the rectal palpation of cows from 42 days of gestation is a safe and reliable method when performed carefully and skillfully. In those cows in which the pregnancy failed, it would probably have occurred irrespective of the procedure used. In a recent controlled, randomised, block-design experiment, it was convincingly demonstrated that the single use of the fetal membrane slip as a diagnostic tool between days 34 and 41 of pregnancy does not affect embryonic/fetal viability, as judged by transrectal ultrasonography at days 45 and 60, in comparison with nonpalpated animals (Romano et al. 2006). Furthermore, in experiments in which attempts have been made to induce abortion by damaging the fetus at rectal palpation, extensive trauma has frequently been necessary (Paisley et al. 1978).

Vaginal Examination

Examination may be manual or visual. In the latter case an illuminated speculum is used. The condition of the vaginal mucous membrane does not afford definite clinical evidence of pregnancy, for the degree of dryness and blanching that occur during the dioestrus are very similar to those of pregnancy. It is to the external os of the cervix that attention is directed. During pregnancy the secretion of the cervical glands becomes gelatinous and tough, forming a plug for sealing the canal. In many cases the seal covers or protrudes from the external os. It has developed by day 60.

Ultrasonographic Methods

Using the ultrasonic fetal pulse detector, which employs the Doppler principle, it is possible to identify the fetal heart from 6 to 7 weeks using a rectal probe. Ultrasonic depth analysers (A-mode) have also been used to detect pregnancy as early as 40 days. Although a level of accuracy of 85% to 95% has been achieved in positively identifying pregnant cows, a large percentage of nonpregnant cows (57%–87%) were incorrectly diagnosed as being pregnant (Tierney 1983). Neither of these two ultrasonic methods would appear to have any advantage over rectal palpation with regard to time of examination or accuracy of the diagnosis.

Real-time B-mode grey-scale ultrasound scanning is the method of choice for the early diagnosis of pregnancy in the cow and has replaced traditional transrectal palpation as the method of choice. Details of the principle of the technique and the equipment available have been described earlier in this chapter. For an excellent description, the reader is advised to consult Ginther (1998) and Boyd and Omran (1991).

Using a transrectal approach to image the uterus and contents, a 7.5 MHz linear transducer is preferable for early pregnancies, whereas a 3.5 to 5.0 MHz transducer is more useful for late pregnancies. After insertion of the transducer, both ovaries should be examined to determine the presence of a CL, followed by the right and left horns. Because of the shape of the probe, the diameter of the rectum and the coiled nature of the horns, it is impossible to scan each horn along its length at the same time, thus cross-sectional images of the horns are frequently identified (Fig. 6.16 C–F). A tentative diagnosis of an early pregnancy can be made on the identification of a nonechogenic (black) area within the lumen of the uterine horn due to the presence of allantoic fluid; however, it must be remembered that fluids other than those associated with pregnancy can give a similar result. Because this occurs first in the horn ipsilateral to the CL of pregnancy, ovarian imaging for the presence of this structure is important. However, the definitive determination of pregnancy is dependent on the identification of an embryo or fetus.

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Using a 7.5 MHz transducer, Boyd et al. (1988) were able to recognise pregnancy as early as 9 days, whereas Pierson and Ginther (1984), using a 5 MHz transducer, were able to do so at 12 and 14 days in heifers before the blastocyst had elongated (see Chapter 4). One has to realise that these very early diagnoses took place under controlled experimental conditions, with animals repeatedly scanned at short intervals. This is very different from the on-farm situation, in which the veterinarian is usually asked to make a conclusive diagnosis upon a single examination. By 17 days, the blastocyst will have elongated and extended into the contralateral horn; this can usually be easily identified with ultrasonography by 26 days. Using a 12-MHz multifrequency linear array rectal probe, diagnosis of pregnancy was made between 18 to 21 days after AI, based on CL blood flow, the size of the CL, and uterine echotexture (Scully et al. 2014).

Experienced persons may accurately diagnose pregnancy around the time of the expected date of return to oestrus in the non-pregnant cyclical animal, but published data indicate that both sensitivity and specificity markedly increase between days 24 and 30 days (Szenci et al. 1998, Romano et al. 2007); after this stage an accurate and rapid diagnosis can be made relatively easily. The data in Table 5.5 illustrates this. Fig. 5.16 shows images at various stages of pregnancy.

Transrectal ultrasonography can also be used to estimate fetal age up to 140 days of gestation (White et al. 1985, Ginther 1998) by the measurement of a number of different fetal dimensions. Of these, the crown–rump length was least frequently capable of being measured, whereas the trunk diameter was the most readily assessed. In addition, it can also be used to determine the sex of the fetus by assessing the relationship between the genital tubercle and surrounding structures. Thus in the male the genital tubercle migrates towards the umbilicus, whereas in the female it migrates towards the tail. The optimum stage for determination is 55 to 70 days (Curran et al. 1989, Stroud 1996) in which they obtained almost a 100% success rate in determining gender. More recently, a study was reported in which the procedure was done from 51 days, although the accuracy, though still very good, was not 100% (99.5% for males and 98.8% for females) (Quintela et al. 2011). It is important to stress that the technique requires considerable experience of transrectal ultrasonography and good equipment. With respect to the plane used for diagnosis, the sagittal view was poor when used for early pregnancy diagnosis; as a consequence, the longitudinal and crosssectional planes were used most frequently. These results demonstrate that ultrasound can be routinely applied under farm conditions to accurately determine the fetal sex in cattle between days 51 and 111 of gestation without apparent influence of cow age, ultrasound scanning plane or fetal sex. However, with the use of sexed sperm in the AI industry, the interest in the use of ultrasound for sexing fetuses has declined.

Optimum Time for Diagnosing Pregnancy

The aim of pregnancy tests is to identify, as early and as accurately as possible, the absence of pregnancy so that steps can be taken to ensure that the cow is served again and thus ensure an optimum calving pattern (see Chapters 25 and 26).

Pregnancy and Its Detection in the Pig

Endocrinology

In the nonpregnant sow the plasma progesterone concentration falls rapidly 15 to 16 days after the previous oestrus, but if conception occurs the CLs persist and the peripheral progesterone concentrations remain elevated at between 30 and 35 ng/ml. Although there is a slight fall to 17 to 18 ng/ml on day 24, the elevated concentration persists for most of gestation, decreasing rapidly just before farrowing. The ovaries and CLs are always necessary for the maintenance of pregnancy. The number of embryos present in utero does not influence the progesterone concentration (Monk & Erb 1974). The minimum concentration of progesterone in the peripheral circulation for the maintenance of pregnancy is about 6 ng/ml (Ellicott & Dziuk 1973); if the levels are lower the pregnancy is lost, but higher levels do not appear to increase embryonic survival.

Total oestrogen concentrations remain fairly constant in plasma during pregnancy, but about 2 to 3 weeks prepartum they begin to increase to about 100 pg/ml, with a sudden surge to values about 500 pg/ml a few days before farrowing. This is followed by a rapid decline after parturition. The early pig blastocyst synthesises substantial amounts of oestrogens (mainly oestrone and oestradiol-17beta), which pass through the uterine wall where they are sulphoconjugated (Heap et al. 1981). These oestrogen metabolites in maternal blood and urine or the deconjugated oestrogens in the faeces reach a temporary peak between 23 and 30 days of gestation (Robertson & King 1974, Choi et al. 1987). This rise can be exploited for the early diagnosing of pregnancy, using either plasma or faecal samples (see later in this chapter).

Methods of Pregnancy Diagnosis

Management Methods

Traditionally, failure to return to oestrus at 18 to 22 days after service or artificial insemination has been regarded as a possible first sign of pregnancy. However, the detection of oestrus can be difficult, and it is time-consuming; even the back pressure or riding test, which is generally accepted to be the most reliable (Reed 1969), is inconsistent. Failure to return to oestrus may be due to a reluctance to show signs, anoestrus or ovarian cysts (see Chapter 22). It is important to know as soon as possible if a sow or gilt is not pregnant, so that she can be served again, treated or culled. A reliable method is also necessary so that breeders can certify that an animal is pregnant before sale. Any technique must be accurate, capable of being used early in gestation and fairly inexpensive.

Clinical Methods

Transrectal Palpation

This method has been described in detail by Meredith (1976) and Cameron (1977). It is dependent upon palpation per rectum of the cervix, uterus and middle uterine arteries. Although the method can provide an immediate result without the need for a specialist and expensive equipment, it is rarely used now. Changes in the middle uterine artery are an important feature of the method; an excellent description is given by Cameron (1977).

Ultrasonographic Methods

The use of the Doppler technique to diagnose pregnancy in the sow (on the basis of detecting a pulsating uterine artery and/or fetal heart beats) was first described by Fraser and Robertson (1968). The earliest diagnosis made using a rectal probe is about 25 days of gestation. The accuracy of the method is reasonable for the diagnosis of pregnant sows (92%–100%), but it is less reliable for nonpregnant sows (25%–100%) (McCaughey 1979). With a single investigation at 5 weeks after mating, a sensitivity of 98.9% and a specificity of 67.2% were obtained (Atkinson et al. 1986),

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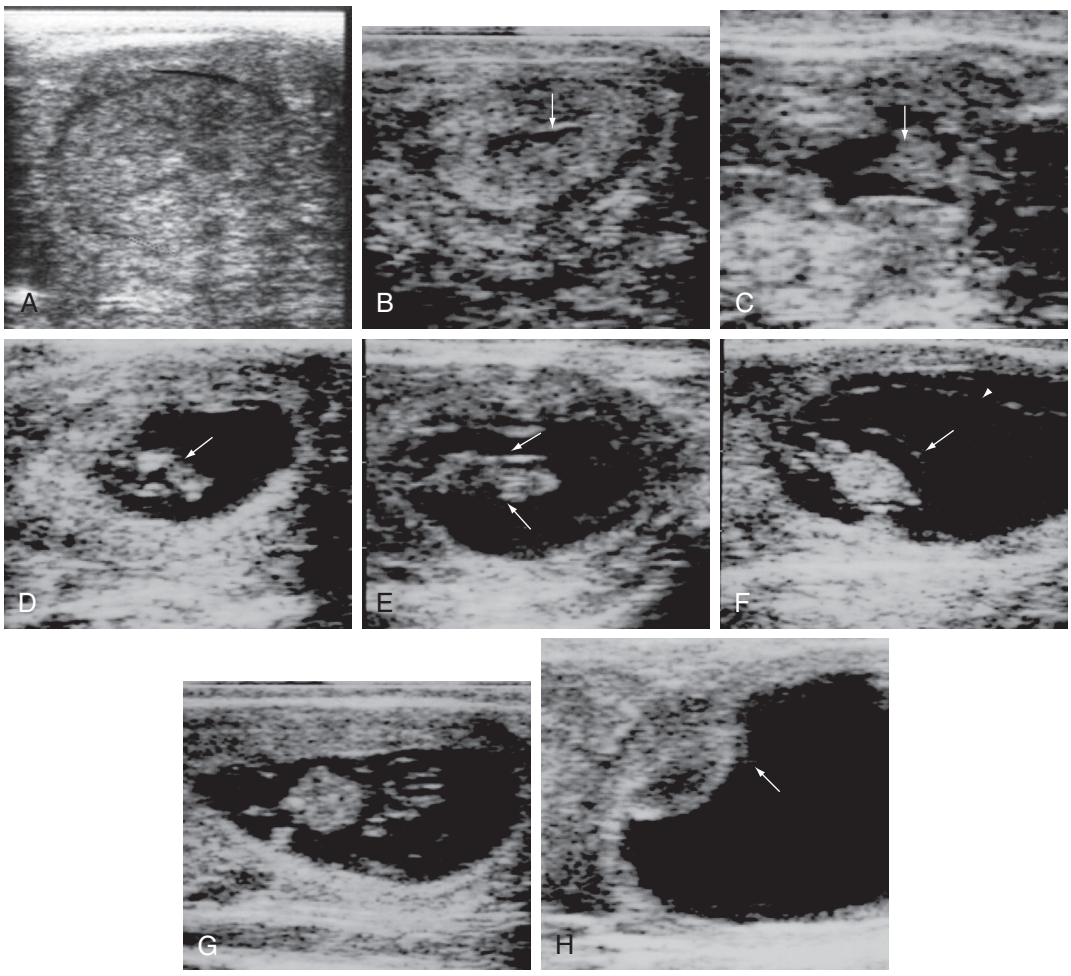

• **Fig. 5.16** Detection of pregnancy in the cow by transrectal ultrasonography. (A) Transverse sectional ultrasonogram of the uterine horn in a nonpregnant cow. Note the alternating bands of anechoic and hyperechoic tissue, which gives a 'rosette-like' appearance due to the endometrial and muscularis layers of the uterine wall. Note that there is no evidence of fluid (which would be anechoic) in the uterine lumen. (B) Transverse sectional ultrasonogram of the uterine horn of a cow at 22 days of gestation. Note that, as in (A), there are alternating bands of anechoic and hyperechoic tissue. The uterine lumen contains fluid, which is anechoic, and just above this *(arrow)* a bright hyperechoic band, which is the allantochorionic membrane. (C) Transverse sectional ultrasonogram of the uterine horn of the same cow as in Fig. 3.16B at 28 days of gestation. As well as the fluid-filled horn, the embryo is now clearly visible *(arrow).* In the real-time image it is possible to see the heart beating. (D) Transverse sectional ultrasonogram of the uterine horn of a cow at 28 days of gestation. Note the embryo's heart *(arrow)* and anechoic allantoic fluid. (E) Transverse sectional ultrasonogram of the uterine horn of a cow at 32 days of gestation. Note that the embryo has visible forelimbs buds *(arrows).* (F) Transverse sectional ultrasonogram of the uterine horn of a cow at 35 days of gestation. The embryo is about 17 mm long. The amnion *(arrow)* and allantochorion *(arrow head)* can be identified. (G) Transverse sectional ultrasonogram of the uterine horn of a cow at 42 days of gestation. Note the fetal body, head and limbs. (H) Transverse sectional ultrasonogram of the uterine horn of a cow at 63 days of gestation showing a placentome *(arrow).* (From Sheldon & Noakes 2002, by permission of *In Practice*, BVA publications.)

and these figures did not improve when testing took place both at 5 and 8 weeks.

Ultrasonic amplitude-depth analysis (A-mode ultrasound) has proved to be more reliable and was the most frequently applied technique until B-mode ultrasonography was introduced. In a study involving 1001 sows using a 2 MHz external transducer probe, Lindahl et al. (1975) reported a 99% accuracy in identifying pregnant sows and a 96% accuracy for nonpregnant sows. These results were obtained between 30 and 90 days of gestation; unreliable results were obtained before 30 days. Taverne et al. (1985a) reported an equally high sensitivity (97.5%) but a much lower specificity (55.8%) when this technique was used between days 31 and 37.

B-mode direct imaging has proved to be very successful in the sow (Martinat-Botté et al. 2000). The transducer probe (or even the entire small-sized scanner) is applied to the abdominal wall of the standing sow about 5 cm caudal to the umbilicus, to the right or the left of the midline and just lateral to the teats, and is directed towards the caudal abdomen; a coupling medium is always required. Pregnancy diagnosis is based on the recognition of nonechogenic irregularly shaped black spots, representing sections through allantoic fluid-filled fetal sacs within the uterus

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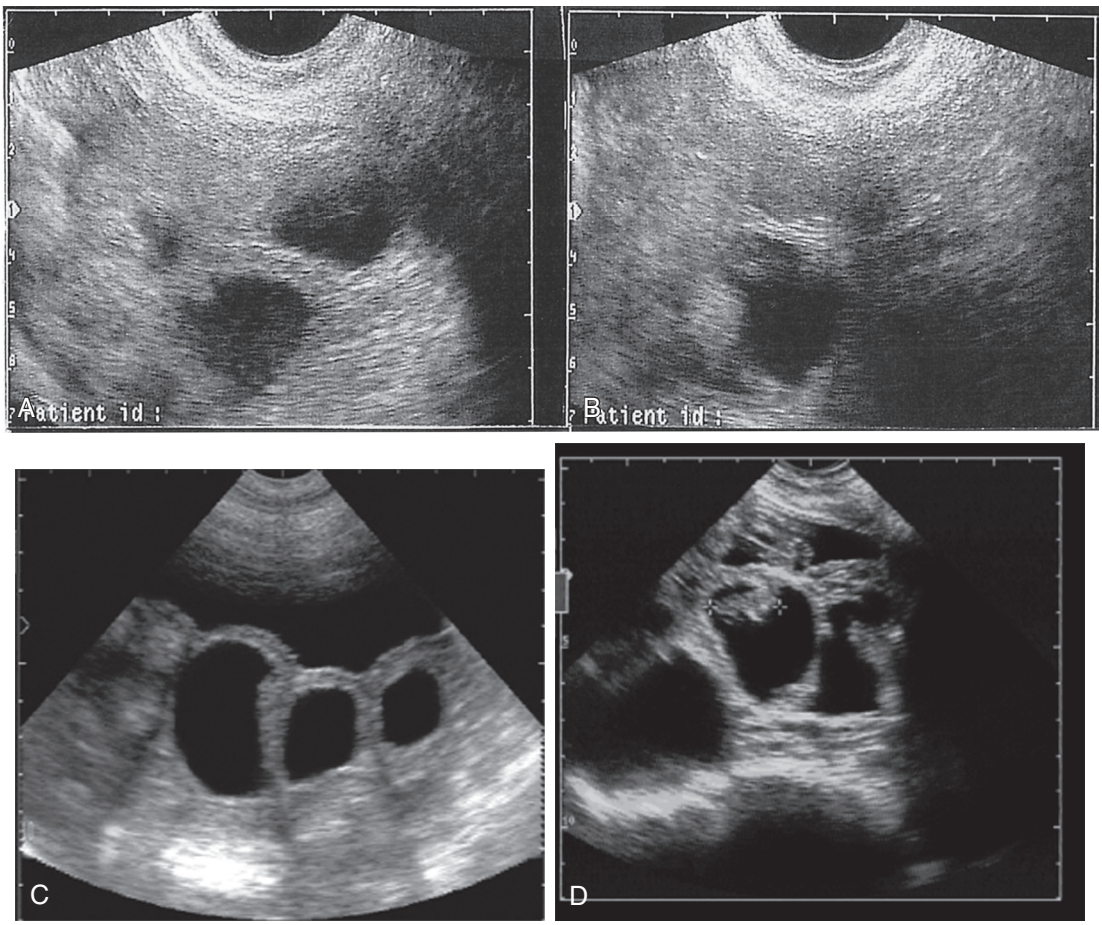

• **Fig. 5.17** Transrectal B-mode (5 MHz transducer) ultrasound images of pregnant sows at 23 days (A–C) and 24 days (D) after service. Black areas represent sections through the allantoic fluid-filled allantoic cavity within the uterine horns in (C) there are three visible below the bladder. In one of these sections in (D) an embryo is visible. (Figs. A and B courtesy of Dr. Roy Kirkwood.)

(Fig. 5.17B and D). Frequently, the embryos can be identified (Fig. 5.17) but are usually not required to reach a positive diagnosis. This implies that accumulation of pathological (mucometra) or physiological (oestrus, semen) fluid within the uterus may result in a false-positive diagnosis. In a study of 145 sows, Inabe et al. (1983) reported a 100% accuracy for the diagnosis of pregnancy from 22 days of gestation. Jackson (1980) reported a 100% accuracy for identifying nonpregnant sows and a 99% accuracy for diagnosing pregnancy from 24 to 37 days of gestation. The small number of errors in this study of 285 sows was due to prenatal death. In a study with 881 animals scanned with a 3.0 MHz linear array transducer between days 24 and 32, Taverne et al. (1985a) also reached 100% sensitivity, but specificity was 90.6%, mainly because of embryonic/fetal death occurring after the day of scanning. A similar conclusion was reached by Szenci et al. (1997) and Vos et al. (1990) when comparing the results of ultrasonography and a faecal unconjugated oestrogen test (see later in this chapter) concurrently performed in the same animals between days 26 and 32.

Transrectal scanning (with an elongated transducer) has also been used (see Fig. 5.17A and C). It usually improves the quality of the ultrasound pictures and enables a slightly earlier diagnosis (Thayer et al. 1985), although after the fourth week, results with a 3.5 and 5.0 MHz were equally accurate, regardless of whether transabdominal or transrectal methods were used (Fraunholz et al. 1989). However, the transrectal approach takes slightly longer and is more difficult to perform, especially when sows are not restrained.

Laboratory Methods

Estimation of Plasma Progesterone. Because there is a decline in progesterone concentrations in the peripheral blood from about day 16 in nonpregnant cyclical animals (see Fig. 5.3) estimation of progesterone concentrations from this time after service is worthwhile. Ellendorff et al. (1976) reported that when sows were bled 16 to 24 days after service a sensitivity of 99.6% and a specificity of 85.3% was achieved. Subsequently, much lower specificities have been reported by several other workers, mainly attributable to late embryonic and fetal mortality of whole litters after the day of testing. However, the biggest problem with this technique remains the difficulty of obtaining blood samples.

Plasma Oestrogen Assay. Robertson et al. (1978) were unable to detect oestrone sulphate in the blood of nonpregnant cyclical sows, yet in pregnant animals it was detectable from day 20 of gestation. Therefore this can be used as a method of diagnosing pregnancy. A small volume of blood sufficient for the assay can be collected from the ear vein; the optimum time for diagnosis, maximum concentrations of oestrone sulphate are present at about 24 to 28 days. Because these oestrogens appear in an unconjugated form in the faeces (Choi et al. 1987), they can be assayed within the same time period. In this way, Vos et al. (1999) obtained a

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sensitivity of 96.5% and a specificity of 93.5%, using an oestrone concentration of 3.65 ng/g faeces as a discriminatory value.

Other Potential Laboratory Methods. In some sows it is possible to detect early pregnancy factors present in the sera of pregnant pigs, particularly by 3 or 4 weeks postmating, which could be detected by the rosette inhibition test. However, the test is not sensitive enough to allow specific diagnosis of early pregnancy in pigs (Grewal et al. 1985; Koch & Ellendorff 1985).

Pregnancy and Its Detection in the Sheep and Goat

Endocrinology

Sheep

In the nonpregnant cyclical ewe, progesterone concentrations in the peripheral blood fall rapidly just before the onset of oestrus (see Fig. 1.30). Following conception, the CL persists and peak dioestrus values are maintained and gradually increase to about 60 days of gestation, when there is a considerable increase, a rise due to the placenta contributing to progesterone production. Levels remain high until the last days of pregnancy, when they decline rapidly to 1 ng/ml at parturition. The concentration of progesterone is significantly higher in multiple pregnancies (Bassett et al. 1969) because it has been calculated that, in late pregnancy, the placenta produces five times as much progesterone as the corpus luteum (Linzell & Heap 1968). Maximum progesterone concentrations in the peripheral blood of ewes with a single lamb were 3.8 ng/ml between days 105 and 110, with twins 5.1 ng/ml between days 125 and 130, and with triplets 9.2 ng/ml between days 125 and 130 (Emady et al. 1974) (Fig. 5.18).

Oestrogen concentrations in the peripheral circulation remain low throughout gestation. A few days before parturition, they start to gradually rise, then suddenly increase to about 400 pg/ml at the time of lambing, followed by a rapid fall (Challis 1971).

Prolactin concentrations fluctuate during pregnancy between 20 and 80 ng/ml towards the end; however, they start to increase and reach a peak of between 400 and 700 ng/ml on the day of lambing (Davis & Reichert 1971, Kann & Denamur 1974).

Placental lactogen has been detected in maternal plasma from 48 days of gestation; it reaches a maximum by about 140 days, gradually decreasing until lambing. It has been identified in trophoblast tissue from 16- to 17-day blastocysts (Martal & Djiane 1977). The role of this hormone is still unclear: it may have a role in the luteotrophic complex of the pregnant ewe and also in controlling fetal growth and mammary development.

Bilateral ovariectomy after 55 days will not result in abortion because by this stage of gestation the placenta has taken over the major role of progesterone production. However, it should be remembered that the CLs persist for the duration of the pregnancy and regress only at the time of parturition (see Chapter 6).

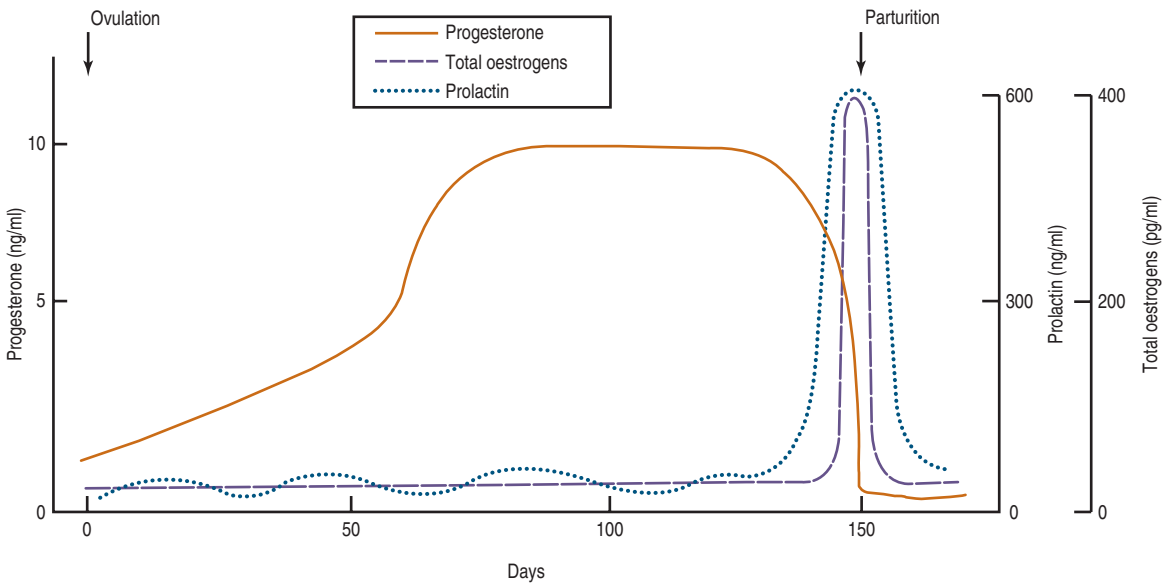
Goat

As in the ewe, progesterone concentrations in the peripheral blood and milk decline around the time of oestrus; thus sampling approximately 21 days after service or artificial insemination will enable a distinction to be made between nonpregnancy and pseudopregnancy or pregnancy. In the latter, they increase until a plateau is reached and then decline rapidly a few days before parturition. Total oestrogens in the peripheral circulation are much higher than those recorded in the ewe. They increase gradually from 30 to 40 days of gestation, reaching a peak value greater than 600 pg/ml just before parturition (Challis & Linzell 1971). Prolactin remains low during pregnancy but rises rapidly just before parturition.

Bilateral ovariectomy at any stage of gestation will result in loss of the pregnancy; thus extraovarian sources would appear to be unable to produce sufficient progesterone for the maintenance of pregnancy.

Methods of Pregnancy Diagnosis in Sheep

There are a large number of published methods of diagnosing pregnancy in the ewe, many of which are historical, and these have been reviewed in detail (Richardson 1972, Karen et al. 2001). The number and variety of methods point to the fact that there was not



• **Fig. 5.18** Schematic representation of the trends in hormone concentrations in the peripheral circulation of the ewe during pregnancy and parturition.

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a simple, accurate and inexpensive clinical method of diagnosing pregnancy in the ewe until the advent of B-mode ultrasonography, which is without doubt the method of choice. Given the rather high fertility of sheep, determination of fetal numbers (for a more efficient use of feedstuffs in late pregnancy and to prevent the occurrence of pregnancy toxaemia), rather than diagnosing the pregnancy itself, appears to offer the main economic benefit of sheep farmers (Russell 1989).

Management Methods

Traditionally, the method used by shepherds is the observation that ewes, which have been marked by a 'keeled' or 'raddled' ram, fail to be marked again within 16 to 19 days. This is a sufficiently reliable sign of pregnancy for most purposes, but subsequent embryonic death will reduce its accuracy, and 20% to 30% of pregnant ewes will show oestrus during early pregnancy. It is important to ensure that the colour of the raddle crayon is changed regularly every 16 days, that it is sufficiently soft to produce a mark and that the colour sequence allows easy colour identification.

Beyond 100 days of gestation the fetus may be palpated through the abdominal wall, and development of the udder is then obvious in primipara. The best way to ballotte the fetus is to have the ewe standing normally and to lift the abdomen repeatedly immediately in front of the udder; the fetus can be felt to drop on to the palpating hand.

Ultrasonographic Methods

The fetal pulse detector (Doppler) has been used to diagnose pregnancy in ewes, and two types of probe are available. The external probe is applied to the skin surface of the abdomen just cranial to the udder. The fleece in this region is sparse and, with transmission gel applied to the end of the probe, it is slowly moved over the surface. The ewe can be restrained either standing or sitting on her haunches. Characteristic sounds indicate the presence of the fetal heart or vessels; the frequency greatly exceeds that of the mother's pulse and heart rate, except in late gestation when the fetal heart rate is reduced. Between 40 and 80 days of gestation the accuracy of detection is no better than 60% (Hulet 1968, Richardson 1972). However, after 110 days, with a reasonable amount of practice, it is nearly 100% accurate, and it takes an average 3 or 4 minutes per ewe to make a diagnosis (Watt et al. 1984).

Using a rectal probe, Lindahl (1971) obtained a sensitivity of 90% or more at the start of the second trimester, whereas Deas (1977) obtained a sensitivity of 82% and a specificity of 91% between days 41 and 60 of gestation. There are several reports giving similar results. The rectal probe is safe and relatively easy to use and requires limited restraint of the ewe. With the fetal pulse detector the diagnosis of false positives should be virtually nil, with the major source of error being the confusion of the maternal pulse sounds with those of the fetus. However, false negatives are always a possibility, especially when testing is taking place too early or too quickly. The external probe can also be used between 80 and 100 days to differentiate between single and multiple pregnancies, although the accuracy in identifying the precise number of fetal lambs is poor.

The B-mode ultrasound (sector) transducer probe, using the transabdominal approach, has proved to be an early, accurate and rapid method of not only differentiating pregnant from nonpregnant ewes but also accurately determining fetal numbers (Fig. 5.19 and Crilly et al. 2017). The cost effectiveness of such a procedure is obvious because it is possible not only to eliminate barren ewes but also to adjust feeding levels to accommodate the number of lambs. Not only does this save on feed cost, but it also reduces the chances of pregnancy toxaemia occurring. Pregnancy can be detected as early as 30 days, although the optimum time to differentiate fetal numbers is 45 to 50 days. White et al. (1984), using a 2.25, 3 or 3.5 MHz transducer, examined a total of 1120 ewes, 36 to 90 days after tupping. The fleece was shorn on the abdomen of each ewe extending some 20 cm cranial to the udder and across the whole width. The ewe was restrained on her back and, using vegetable oil as a coupling medium, the abdomen was scanned. A positive diagnosis of pregnancy, based upon the imaging of a fluid-filled uterus and placentomes, could be made quite quickly. Care was taken to examine the limits of the uterus so that fetal numbers could be detected accurately. An experienced person could examine on average 75 ewes per hour. It is also possible to examine the ewe in a sitting position. An experienced person was over 99% correct in differentiating pregnant from nonpregnant ewes and 98.9% in identifying fetal numbers (Fowler & Wilkins 1984). The most common source of error was failing to identify the third fetus in ewes with triplets. Inexperienced persons soon developed a high level of accuracy, especially at differentiating between pregnant and nonpregnant ewes. Commercially, B-mode ultrasound scanning is done with the ewe placed in a rotating handling system to allow easy movement of the transducer over the (shorn) area in front of the udder and over the uterus (White et al. 1984, Russell 1989), or with the animal in standing position in an elevated restraining crate, with the transducer probe placed on the hairless area just cranial and lateral to the udder (Taverne et al. 1985b). Yet, with the animals in the supine position and without shaving, Karen et al. (2006) reported rather low accuracies for discriminating between single and multiple pregnancies during a field study with Awassi x Merino sheep between days 43 and 87 of gestation. Transrectal scanning has also been explored in sheep, although often with smaller numbers of animals (Buckrell et al. 1986, Garcia et al. 1993, Karen et al. 2004) and allows for a somewhat earlier diagnosis.

Laboratory Methods

Milk and Plasma Progesterone. Pregnancy can be diagnosed on the fact that, in the pregnant ewe, the CLs persist and hence peripheral progesterone concentrations will remain elevated at 15 to 18 days after mating. The presence of a persistent CL in the absence of a pregnancy may occur, resulting in an incorrect result. Furthermore by using a 2.5 ng/ml discriminatory value using an enzyme immunoassay for samples collected 19 days after intravaginal progestogen sponge removal, Boscos et al. (2003) obtained a sensitivity of 98.3% and a specificity of 85.5%. Plasma levels, when measured using radioimmunoassay, did not prove accurate in discriminating between single and multiple pregnancies in samples taken between days 43 and 87 after artificial insemination in synchronised ewes (Karen et al. 2006).

Pregnancy Specific Proteins. As in cattle, the binucleate cells of the ovine placenta produce pregnancy-specific proteins (ovPSPBs), also known as pregnancy-associated glycoproteins (ovPAGs) (Ward et al. 2002). They are detectable in the maternal circulation from week 3 of gestation until 2 to 3 weeks after lambing. With antibodies to bovine PSPB, Ruder et al. (1988) obtained a sensitivity of 100% and a specificity of 90% in a pregnancy test with blood samples taken between days 35 and 106. By using a heterologous, double antibody radioimmunoassay against a bovine PAG 67 kDa subunit, Karen et al. (2003) obtained high sensitivity and specificity from days 22 of pregnancy onwards. Measurements of PAG were unreliable for an accurate prediction of fetal numbers (Vandaele

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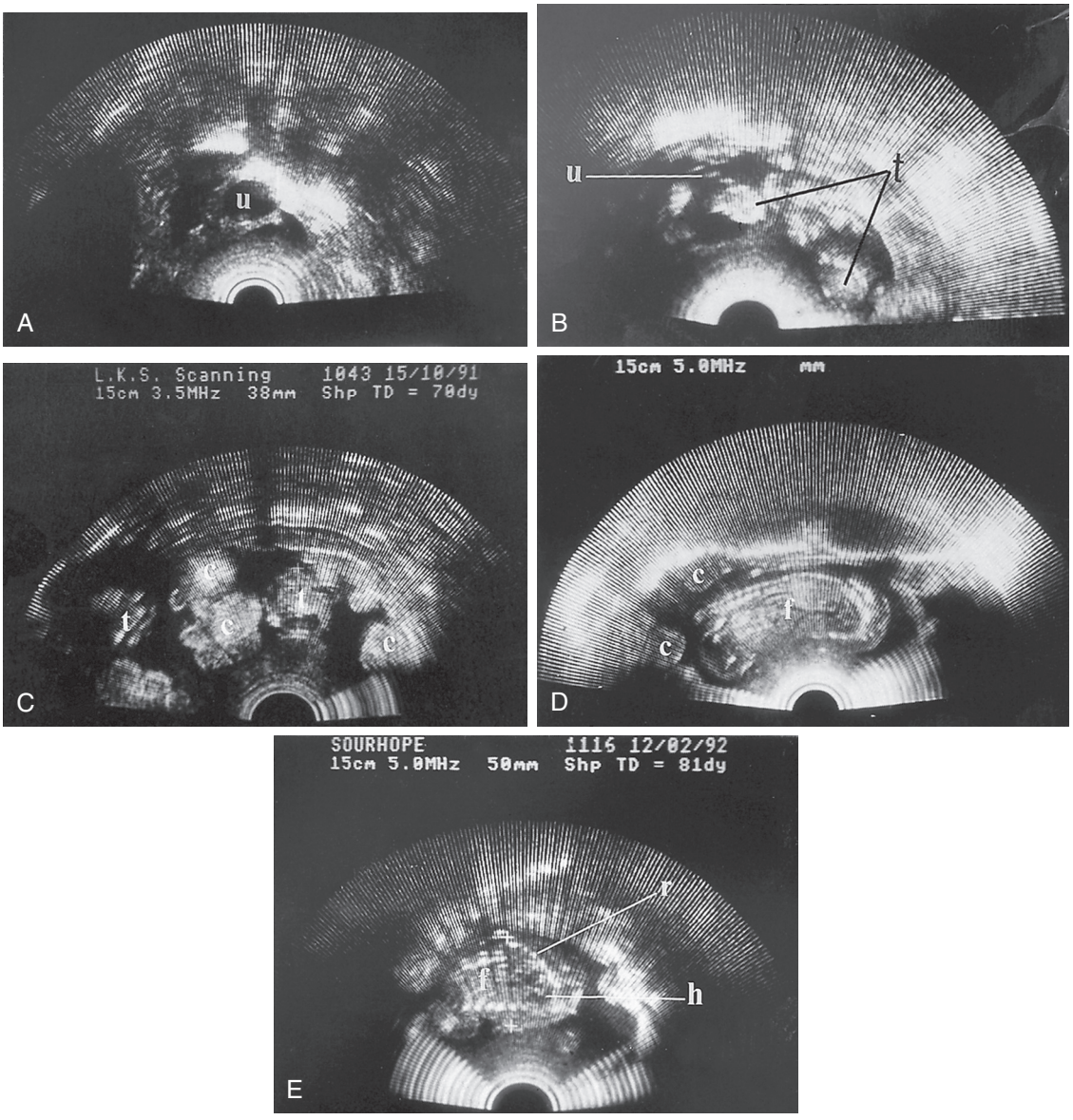

• **Fig. 5.19** Transabdominal B-mode ultrasound images of a ewe's pregnant uterus and its contents using 3.0 or 5.0 MHz sector transducer. (A) At approximately 35 days of gestation. At this stage it is possible to identify fluid-filled sections of the uterine horns *(u).* In the absence of an embryo or fetus it is only indicative of a possible pregnancy. (B) At approximately 55 days of gestation. Note twin fetuses *(t)* surrounded by nonechogenic (black) amniotic fluid. The umbilical cord can be seen attached to the left fetus *(u).* (C) At approximately 70 days of gestation. Note twin fetuses *(t)* and cotyledons *(c).* (D) At approximately 80 days of gestation. Note the large single fetus *(f),* in which the spine can be identified; also cotyledons *(c).* (E) At approximately 81 days of gestation. Note the single fetus (f) with ribs *(r)* and heart *(h).* (Courtesy of Dr. P. J. Goddard.)

et al. 2005, Karen et al. 2006). More recently, early pregnancy diagnosis in sheep, using near-infrared spectroscopy (NIRS) on blood plasma samples taken at day 18 and 25 after AI, has been described (Andueza et al. 2014); the results were very accurate compared to those obtained by assaying plasma P4 and PAG (Table 5.6).

Methods of Pregnancy Diagnosis in the Goat

Many of the methods which have been described above for the ewe are also applicable to the goat. Using the fetal pulse detector, a reliable diagnosis of pregnancy is possible at 50 days using an abdominal probe and at 25 days with a rectal probe. Excellent results have also been obtained with B-mode direct imaging with rectal or abdominal probes from about 30 days of gestation (Lavoir & Taverne 1989, Hesselink & Taverne 1994, Gonzalez et al. 2004, Holtz 2005), and the method also enables the accurate determination of fetal numbers (Fig. 5.20A). Scanning at an early stage after mating or artificial insemination (before day 30) may result in a false-positive diagnosis if the presence of fluid in the uterus of pseudopregnant does (mucometra/hydrometra; see Fig. 5.20B) is mistaken for a sign of pregnancy (Hesselink and Taverne 1994, Lopes Juacutenior et al. 2004). Transrectal ultrasound is an

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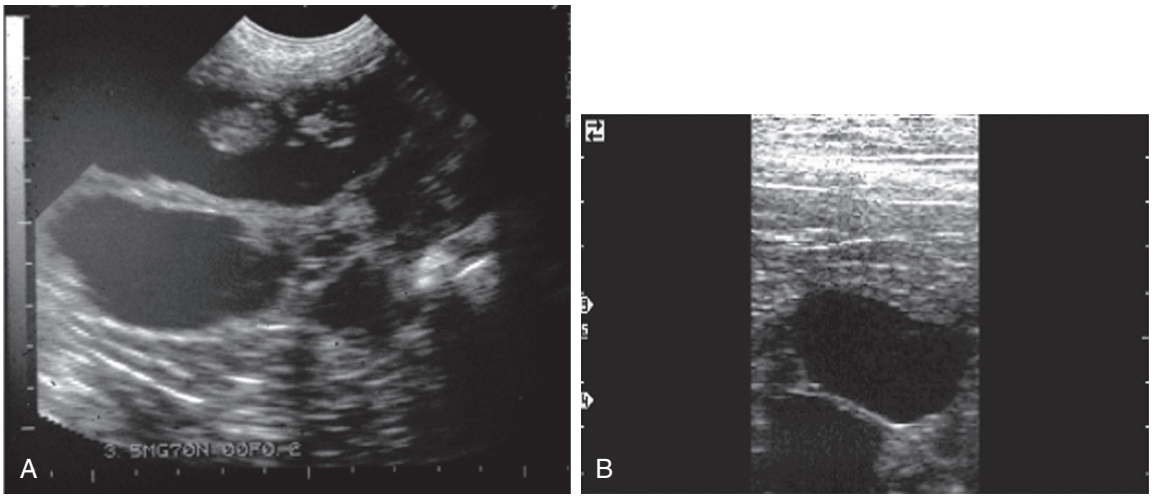
TABLE 5.6 Sensitivity, specificity, positive predictive value (PPV), and negative predictive value NPV) of NIRS* compared with the plasmatic PAG method (≥0.8 ng/mL) at days 18 and 25 after AI and P4 method (≥0.5 ng/mL) at day 18 after AI**

Method	Sensitivity	Specificity	PPV	NPV
Day 18				
NIRS	98.9 (89/90)[a]	100 (98/98)[a]	100 (89/89)[a]	99.0 (98/99)[a]
PAG	32.2 (29/90)[b]	100 (98/98)[a]	100 (29/29)[c]	61.6 (98/159)[b]
P4	100 (90/90)[a]	84.7 (83/98)[b]	85.7 (90/105)[b]	100 (83/83)[a]
Day 25				
NIRS	100 (90/90)[a]	100 (29/29)[a]	100 (90/90)[a]	100 (29/29)[a]
PAG	100 (90/90)[a]	100 (29/29)[a]	100 (90/90)[a]	100 (29/29)

*NIRS: Normal inactivated rabbit serum
**PAG: Pregnancy-associated glycoprotein
From Andueza et al. 2014.



• **Fig. 5.20** Transabdominal ultrasound images of (A) a pregnant and (B) a pseudopregnant goat. Note the presence of an embryo with head, front legs and rump in the section through the uterus close to the abdominal wall at the upper part of the picture. In the pseudopregnant goat two sections through the fluid-filled uterine horns are visible, closely resembling the picture of an early pregnancy.

effective method of early pregnancy diagnosis and determining fetal number at days 24 to 29 and days 31 to 36, respectively, with comparable accuracy with the transabdominal approach days 39 to 51. The breed and age of does might influence the accuracy of ultrasonography for pregnancy diagnosis and fetal numbers estimation (Karen et al. 2014). Ultrasonographic fetometry has been used to estimate gestational age in goats (Karen et al. 2009).

The milk progesterone test has been used extensively because most parous goats are lactating at the time of mating. Whole milk samples are collected from the bucket after thorough mixing on or about the day of mating and in the absence of return to oestrus, 22 and 26 days later (Holdsworth & Davies 1979). The accuracy for identifying the absence of pregnancy was 100%; however, some false-positive results were obtained due to pseudopregnancy, ovarian cysts and elevated progesterone values at oestrus. However, given the advantages of B-mode ultrasonography, it seems unlikely that the method will ever become routine in goat husbandry.

Oestrone sulphate is produced by the placentomes, and its presence in plasma, milk, or faeces is a positive indication of pregnancy, thus enabling differentiation from pseudopregnancy. Although it has been shown that oestrone sulphate concentrations from 30 days of gestation exceed those in nonpregnant individuals (Heap et al. 1981), the earliest optimum time is 50 days or later after service (Chaplin & Holdsworth 1982).

Determinations of PSPB (Humblot et al. 1990) or PAGs (Sousa et al. 1999, Gonzalez et al. 2004) in plasma have also been used for pregnancy diagnosis in goats and appeared accurate from 3 to 4 weeks of pregnancy onwards. Because the proteins also appear in the milk, this might become an attractive test for dairy goat farms.

Pregnancy and Its Detection in the Dog

Endocrinology

The bitch has a prolonged luteal phase, with the CLs remaining active for 65 to 75 days in the nonpregnant animal. The uterus does not appear to have a role in influencing the life span of the CL in the nonpregnant state (Okkens et al. 1985). The ovaries of the bitch are necessary for the maintenance of pregnancy; their

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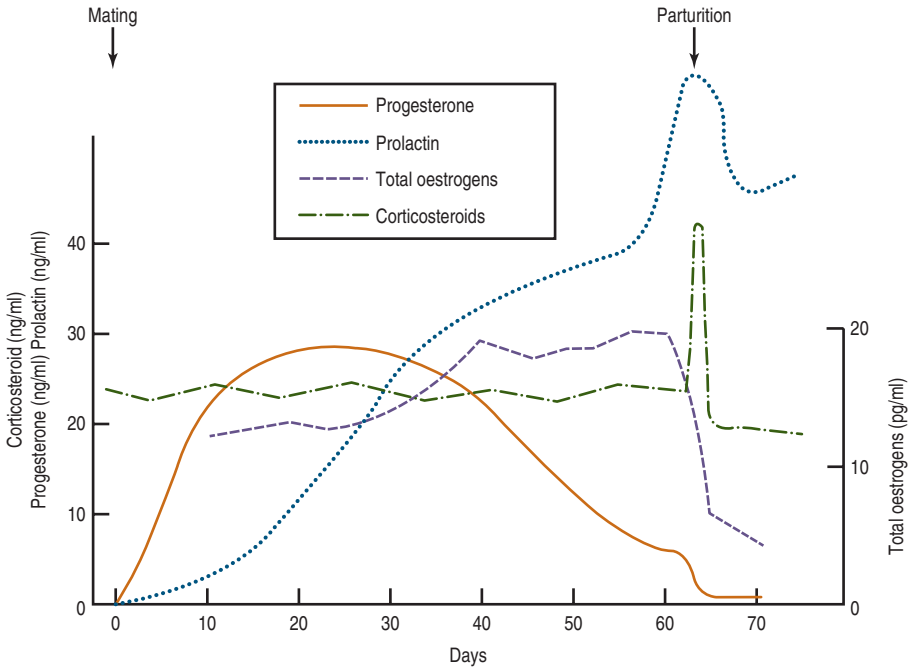

• **Fig. 5.21** Schematic representation of the trends in hormone concentrations in the peripheral circulation of the bitch during pregnancy and parturition.

removal even as late as 56 days resulted in abortion (Sokolowski 1971). Progesterone concentrations in the peripheral circulation of pregnant bitches are similar to those of nonpregnant individuals and, for this reason, unlike in other species, cannot be used to diagnose pregnancy. Concannon et al. (1975) obtained mean maximum values of 29 ng/ml for pregnant and of 27 ng/ml for nonpregnant bitches. However, there was a lot of individual variation, with peak values obtained between 8 and 29 days after the LH peak in pregnant bitches and between 12 and 28 days in nonpregnant bitches. From about 30 days of gestation, there is a gradual decrease in progesterone so that by about day 60 values of 5 ng/ml are obtained, followed by a sudden decline just before parturition, to zero just after. In the nonpregnant bitch there is no rapid fall; low levels of progesterone persist. The number of days in which values greater than or equal to 1 ng/ml were obtained were 68 days in nonpregnant compared with 63.8 days in pregnant bitches (Concannon et al. 1975).

Peripheral plasma oestrogen (Fig. 5.21) values are slightly, but not significantly, higher in the pregnant bitch than in the nonpregnant bitch, with some evidence of an increase at the time of implantation (Concannon et al. 1975). They remain fairly constant, though variable, during the rest of gestation (Luz et al. 2006), before declining significantly to basal nonpregnant values by the day of parturition (Baan et al. 2008).

Prolactin is the main luteotrophic hormone, especially during the second half of gestation (Okkens et al. 1990, Onclin et al. 2000). Although prolactin concentrations increase during the first half of the luteal phase in both pregnant and nonpregnant bitches, there is a much greater rise in the second half of the former. The gradual rise during pregnancy ends with a sudden surge during the rapid decline in progesterone that occurs 1 to 2 days before whelping (De Coster et al. 1983, McCann et al. 1988, Verstegen-Onclin & Verstegen, 2008).

Relaxin has been detected in the peripheral circulation of pregnant Labrador and Beagle bitches at 20 to 30 days of gestation, reaching maximum concentrations around 5 to 7 weeks; it is absent in nonpregnant bitches at all stages of the reproductive cycle (Steinetz et al. 1989). The main source of relaxin appears to be the placenta (Klonisch et al. 1999), but the ovaries also contribute because serum levels decreased after ovariectomy in pregnant dogs (Steinetz et al. 1989). Significantly lower plasma relaxin levels have been found in pregnant bitches with a high incidence of embryonic/fetal death (Gunzel-Apel et al. 2006).

The average gestation length in the bitch is normally quoted as 63 to 64 days, but the interval from first mating to whelping can vary from 56 to 71 days. However, if gestation length is measured from the time of the preovulatory LH peak, it is very constant at between 64 and 66 days (Concannon et al. 1983). When the preovulatory increase of plasma progesterone was used as a guide for the optimum time of mating, an average gestation length of 61.4 days was calculated (Okkens et al. 2001). In a recent retrospective study, breed and litter size, but not age and parity, appeared to influence gestation length in dogs (Eilts et al. 2005).

Methods of Pregnancy Diagnosis

Because the bitch is not polycyclic, pregnancy cannot be anticipated by a failure to return to oestrus. It is generally accepted that the period of oestrus ends more abruptly when fertilisation has occurred, although the reason for this has remained unclear. One of the problems in diagnosing pregnancy in the bitch is that overt pseudopregnancy is rather common, the intensity of the signs varying from individual to individual.

The deposition of abdominal and subcutaneous fat during pregnancy is often marked. This is to support the demands of the subsequent lactation because it is generally lost again during the period of nursing. The gravid uterus and its contents cause no appreciable increase in body weight during the first 5 weeks. From this point, body weight rapidly increases according to the number of fetuses. The increase will vary from 1 kg in a 5 kg bitch

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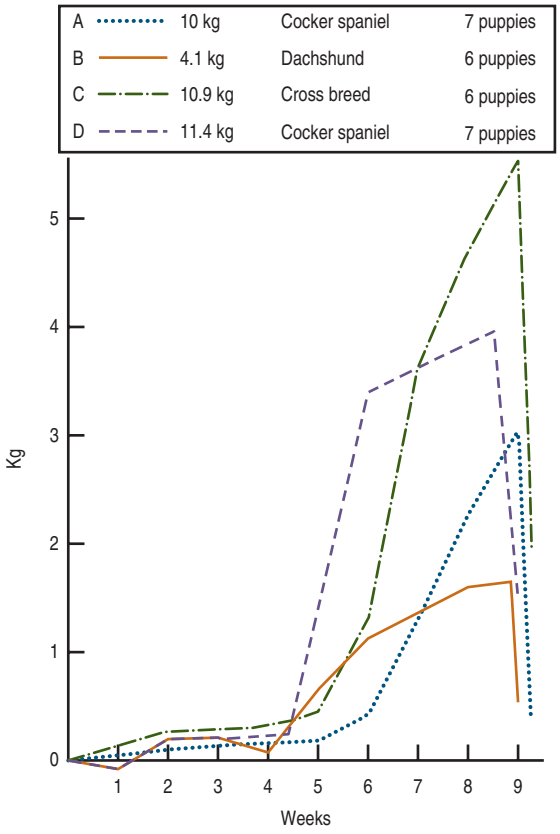
to 7 kg or more in one of 27 kg, but by the time the increase in body weight has occurred, there are other very definite signs of pregnancy (Fig. 5.22). When there are a large number of fetuses, abdominal distension becomes progressive and obvious from the fifth week onwards, but in animals gravid with one or two fetuses only, particularly when the bitch is large or very fat, distension may not be noticeable.

There are several causes of abdominal distension in the bitch that must be differentiated from pregnancy. The most important is pyometra (see Chapter 33); others are ascites, peritonitis with effusion, splenic enlargement, and neoplasia of the liver abdominal lymphatic glands or uterus and are less common.



• **Fig. 5.22** Increase in body weight in bitches during pregnancy.

Clinical Methods

Mammary Glands. Characteristic changes occur in the mammary glands. Unfortunately, similar but less definite changes may occur during pseudopregnancy. These changes are more easily recognised in primigravidae. At about day 35, in unpigmented skins, the teats become bright pink in colour, enlarged and turgid, and they protrude. This condition persists until about day 45, when the teats become larger still but softer and tumefied; they may become pigmented. Appreciable hypertrophy of the glands commences at day 50 and progresses until, at term, the mammary glands comprise two parallel, enlarged and oedematous areas with a depression between them, extending from the pelvic brim to the cranial thorax. A watery secretion can generally be expressed from the teats 2 to 3 days before parturition; however, the onset of milk secretion coincides with parturition. In multigravidae, mammary hypertrophy commences about 7 days before term, and in some cases milk can be expressed from the teats several days before parturition.

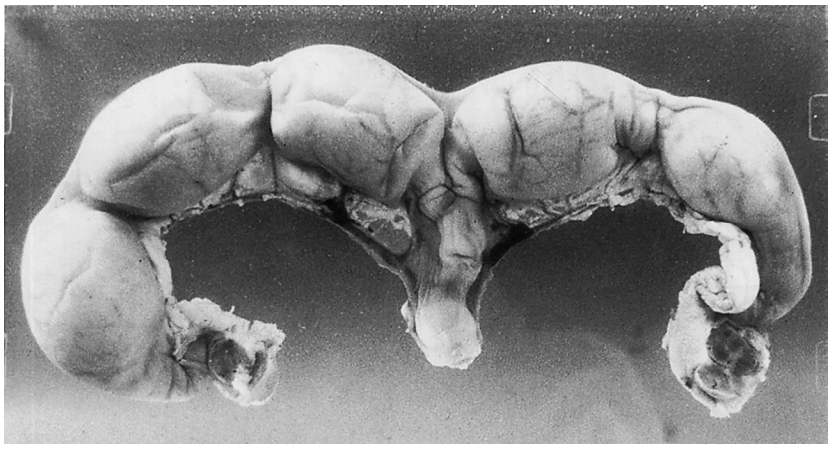
Abdominal Palpation

The ease and accuracy of abdominal palpation will depend upon the following factors:

- The size of the animal: the smaller, the easier
- Her temperament: whether palpation is resisted
- The period in gestation at which examination is made
- The number of fetuses in utero
- Whether the bitch is of normal size or grossly obese

Days 18 to 21. At this stage the embryos represent a series of tense, oval distensions in the horns, about 12 mm long by 9 mm broad. In small bitches that can be readily manipulated, it may be possible to estimate approximately the number present. Those situated in the caudal parts of the horns are most easily felt if there are only one or two; if they are located cranially, they may not be identified. In large or fat bitches it is unlikely that the conceptuses will be detected at this stage. Care must be taken not to confuse the conceptual swellings with faeces in the colon.

Days 24 to 30. This is the optimum period for the early diagnosis of pregnancy. By day 24 these distensions have become spherical in outline, from 6 to 30 mm in diameter. They remain tense and are easily recognised (Fig. 5.23). Sometimes, there is variation in size, the caudal ones being rather smaller than the cranial ones, but impending late embryonic or fetal mortality may also have caused a size difference. The embryonic units maintain this spherical form until about day 33.



• **Fig. 5.23** Uterus and ovaries of a bitch, pregnancy of about 30 days. Note five conceptual swellings.

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Days 35 to 44. The constricted portions of the horns between the embryonic units become progressively dilated; the distensions become elongated and lose much of their tenseness. At this time the uterus comes into contact with the abdominal wall and, in the bitch pregnant with many fetuses, the abdominal distension is starting to become obvious. Nevertheless, palpation of the fetuses themselves is not yet possible. As the uterus itself has lost much of its tension and the amount of fetal fluids has reached its maximum volume, a positive diagnosis may be difficult, particularly in those pregnant with only one or two pups.

Days 45 to 55. During this stage, increase in the size of the fetuses is rapid. At day 45 it may be possible to detect the caudally situated ones between the fingers; in a 9 kg bitch they are approximately 63 mm long and 12 mm broad. It is during this stage that the disposition of the uterus in the abdominal cavity changes. In animals pregnant with many fetuses, each horn represents an elongated cylinder, 38 to 51 mm in diameter and 228 to 300 mm long. Caudally, they extend into the uterine body, which has by this time become dilated. Each horn is in two segments: the caudal, which lies on the abdominal floor and passes forwards to the margins of the liver; and the cranial, which lies dorsal and lateral to it, with its long axis directed backwards towards the pelvis. In the last stages the uterus almost entirely fills the abdomen.

Days 55 to 63. During this period there should be no difficulty in diagnosing pregnancy provided the bitch readily accepts palpation of the abdomen. The size of the fetuses is such that they can readily be detected. High in the flank, the one occupying the apex of the horn will be felt, whereas in the midline just in front of the pelvic brim is the one with its extremity in the uterine body. If manipulation is resisted, digital examination per rectum is helpful. The bitch's foreparts should be raised, and the uterus pressed backwards towards the pelvic inlet by pressure on the abdomen. The presented part of the caudal fetus will be detected beneath the finger. In big or fat bitches pregnant with only one or two, doubt may still exist although by this time the mammary glands afford valuable confirmatory evidence.

The accuracy of abdominal palpation is sometimes questioned, particularly in differentiating pregnancy from overt pseudopregnancy. Allen and Meredith (1981) and Taverne et al. (1985c) have reported on the accuracy of this method. The former authors found that, although pregnancy was detected at 21 to 25 days of gestation, the accuracy was only 52% compared with 87% from 26 to 35 days. However, in the case of the correct diagnosis of bitches that did not subsequently whelp, the accuracies were 92% and 73%, respectively, for the same gestational ages. The optimum time for accuracy is between 26 and 35 days of gestation. Taverne et al. (1985c) concluded, on the basis of 91 investigations between days 25 and 35 after first mating, that sensitivity of abdominal palpation (by an experienced clinician) was 90% and specificity 91%. These accuracies were only slightly lower than the ones obtained with transabdominal ultrasonography (with a 3 MHz transducer) in the same period. Small litters appeared to be the major cause of false-negative diagnoses with both techniques. The authors suggested that it is worthwhile for small animal clinicians to practise the technique of abdominal palpation. It is important for a high level of accuracy that the interval from mating to examination is known because assessment of whether a bitch is pregnant or not is dependent upon the identification of conceptual swellings of a size comparable with the gestational age of the bitch.

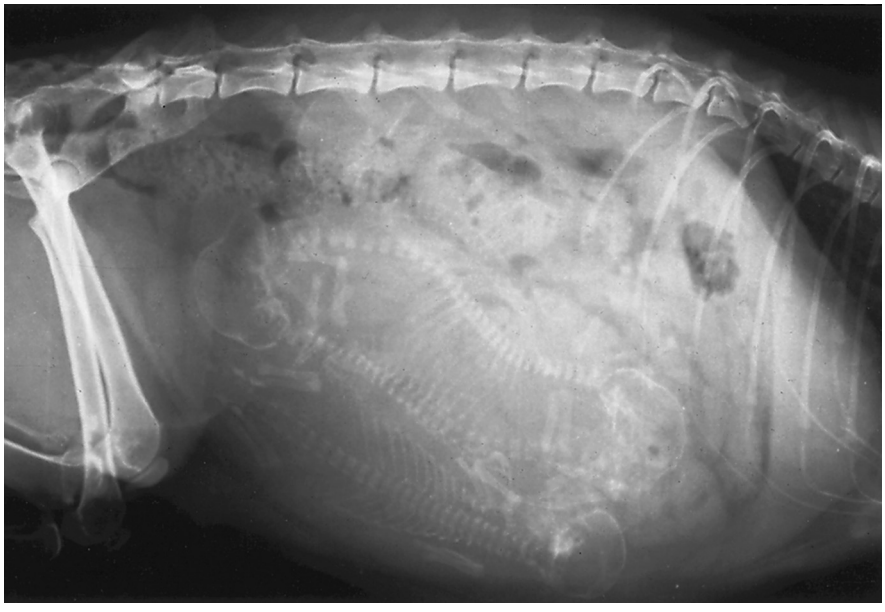
Radiography

Radiography is a particularly useful diagnostic aid in the terminal stages of pregnancy, especially in the obese dog in which a differential diagnosis from pseudopregnancy is required, or in bitches with a presumed small number of fetuses that may suffer prolonged gestation. It may also be valuable in dystocia cases to disclose the presence of retained puppies and the disposition of a presenting puppy, although ultrasonography will usually be the technique of first choice in such cases.

At the end of 7 weeks it is normally possible to identify the whole fetal skeleton. At this stage radiography is the preferable and most accurate technique to count fetal numbers. The accuracy of radiographic diagnosis is very much dependent upon the quality of the radiograph that is obtained (Fig. 5.24).

Ultrasonographic Methods

Using the Doppler method with an external transducer placed on the abdominal wall adjacent to the mammary glands, fetal heart



• **Fig. 5.24** Radiograph of the abdomen of a pregnant bitch near term. Note the fetal skeletons.

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sounds were detected as early as 29 days of gestation (Helper 1970), although in all the 25 bitches confirmed to be pregnant, the fetal hearts were heard by 32 days; they were consistently twice the maternal heart rate. Riznar and Mahek (1978) found that the earliest time a positive diagnosis could be made was at 44 days. They stressed its value in late gestation, 61 to 70 days after mating, especially as a method of confirming the presence of live or dead puppies. Using two different instruments, Allen and Meredith (1981) found a low level of accuracy between 25 and 35 days of gestation, which improved with advancing gestational age to reach 100% with one instrument between 43 and 64 days.

A-mode ultrasound depth analysers have been employed to diagnose pregnancy using an external transducer probe. Smith and Kirk (1975) were able to detect pregnancy as early as 18 days after mating; these authors stressed the importance of not scanning too far caudally because of the chance of reflections from a full urinary bladder. Using a similar instrument with a 2.25 MHz transducer probe, the earliest correct positive diagnosis of pregnancy was made at 26 days (Allen & Meredith 1981). From 32 days to term a level of accuracy of about 90% was obtained for the correct diagnosis of pregnant and nonpregnant bitches.

As with other domestic species, B-mode ultrasonography is now the most frequently used method of diagnosing pregnancy in the bitch (Fig. 5.25). In addition, the technique has been widely explored to predict the day of whelping in bitches with an unknown mating date (Son et al. 2001, Kutzler et al. 2003 Luvoni & Beccaglia 2006). With the development of improved transducers, it became a highly accurate diagnostic tool not only for pregnancy diagnosis, but also for the study of pregnancy failure (England & Russo 2006). The initial studies reported in the literature mainly used low-frequency transducers. Using a 2.4 MHz linear array probe placed on the abdominal wall, positive identification of pregnancy at a high level of accuracy was achieved 28 days after natural mating or artificial insemination (Bondestam et al. 1984). Of 77 bitches that whelped, only one was incorrectly diagnosed as being nonpregnant, an accuracy of 99.3%, and she expelled a macerated fetus, whereas all 58 that were diagnosed as nonpregnant failed to give birth to puppies. In a study including some 135 bitches, investigated between days 20 and 49 after first mating, Taverne et al. (1985c) found a sensitivity of 92.9% and a specificity of 96%. Early identification of the conceptus has been reported at 14 days (Tainturier & Moysan 1984) and 21 days (Taverne 1984). However, the former workers suggested that it was preferable to wait until 20 days.

The accurate estimation of litter size has proved to be difficult, especially in larger breeds of dog. A figure of 40% accuracy at 29 days after mating and 83.3% accuracy from 50 days to term has been reported (Bondestam et al. 1984), with overestimation of small litters and underestimation of large litters.

For merely diagnosing pregnancy it is generally unnecessary to clip the hair of dogs, even in those breeds with long coats, provided that the hair is parted and plenty of coupling gel is used. Many owners do not object to the clipping of the hair between the two rows of teats, enabling more clear and detailed imaging through the midventral abdominal wall.

Laboratory Methods

Measurement of Serum Proteins. Gentry and Liptrap (1981) observed a threefold rise in serum fibrinogen concentrations during pregnancy, with peak values occurring 4 to 5 weeks after mating. Because this phenomenon did not occur at the corresponding stage of the luteal phase in nonpregnant bitches, it can be used as a method of detecting pregnancy. Eckersall et al. (1993) have reported an acute phase response in pregnant bitches as demonstrated by the rise in serum C-reactive protein (CRP) in midgestation. More recently, significant differences have been reported between pregnant and nonpregnant bitches for a wide range of serum acute-phase proteins (Vannucchi et al. 2002), and the authors also suggested that determination of some of these proteins might be useful for (early) pregnancy diagnosis. They are likely to be produced in response to the implantation of the embryo causing tissue damage. However, precise mating dates are required for these tests, and there is always the danger that false positives may arise if there is infection and inflammation elsewhere.

Measurement of Hormones in the Peripheral Circulation. Whereas persistent elevated progesterone concentrations in the peripheral circulation are used to detect pregnancy in the polyoestrous species, they are of no value in the bitch because of the prolonged luteal phase (pseudopregnancy) in nonpregnant animals. Yet, the initial rise in preovulatory progesterone concentrations appeared to be an accurate predictor for the parturition date (Kutzler et al. 2003). The canine placenta does not produce gonadotrophic hormones, but it does produce relaxin (Steinetz et al. 1987, 1989, Klonisch et al. 1999). Peripheral plasma concentrations are elevated from 20 to 30 days of gestation, whereas they are not in the nonpregnant animal; pregnancy detection kits are now commercially available.

Pregnancy and Its Detection in the Cat

Ovulation occurs 23 to 30 hours after mating (Concannon et al. 1980), and serum progesterone concentrations rapidly increase from the baseline of under 10 nmol/l to reach peak values of around 100 nmol/l between the first and fourth weeks of pregnancy (Verhage et al. 1976) (Figs 5.26 and 5.27).

Cats are unusual in that queens may continue to display oestrous behaviour and accept mating even though ovulation has occurred, and there is significant production of progesterone (Stabenfeldt 1974). This evidently favours the occurrence of superfecundation, the presence of offspring from more than one sire in a single litter (see Chapter 9).

At 3 to 4 weeks of pregnancy hyperaemia of the teats occurs. This is particularly prominent in maiden queens. It is a progesterone-dependent phenomenon and is also seen in pseudopregnancy. There is conflicting evidence concerning the relative roles of the CLs and the placentas in the synthesis of progesterone during pregnancy. Scott (1970) and Gruffydd-Jones (1982) have reported the maintenance of pregnancy, following ovariectomy after 45–50 and 49 days respectively when 3-beta-hydroxysteroid dehydrogenase activity, an enzyme essential for progesterone synthesis, is present in the feline placenta (Malassine & Ferre 1979). Yet Verstegen et al. (1993a) have reported abortion following ovariectomy at 45 days of gestation. Progesterone concentrations gradually fall from their peak values during the first month of pregnancy, the fall becoming precipitous during the last 2 days prior to parturition. In many, but not all, queens there is a slight preparturient increase in oestrogen concentrations, but this declines just before parturition.

Relaxin is produced by the placenta during pregnancy and is thought to contribute to its maintenance by inhibiting uterine activity. It appears during the third week of pregnancy, with concentrations declining just before parturition (Stewart & Stabenfeldt 1985). Prolactin is considered a luteotrophic hormone during the last third of pregnancy (Jöchle 1997), and concentrations decline as weaning takes place (Banks et al. 1983).

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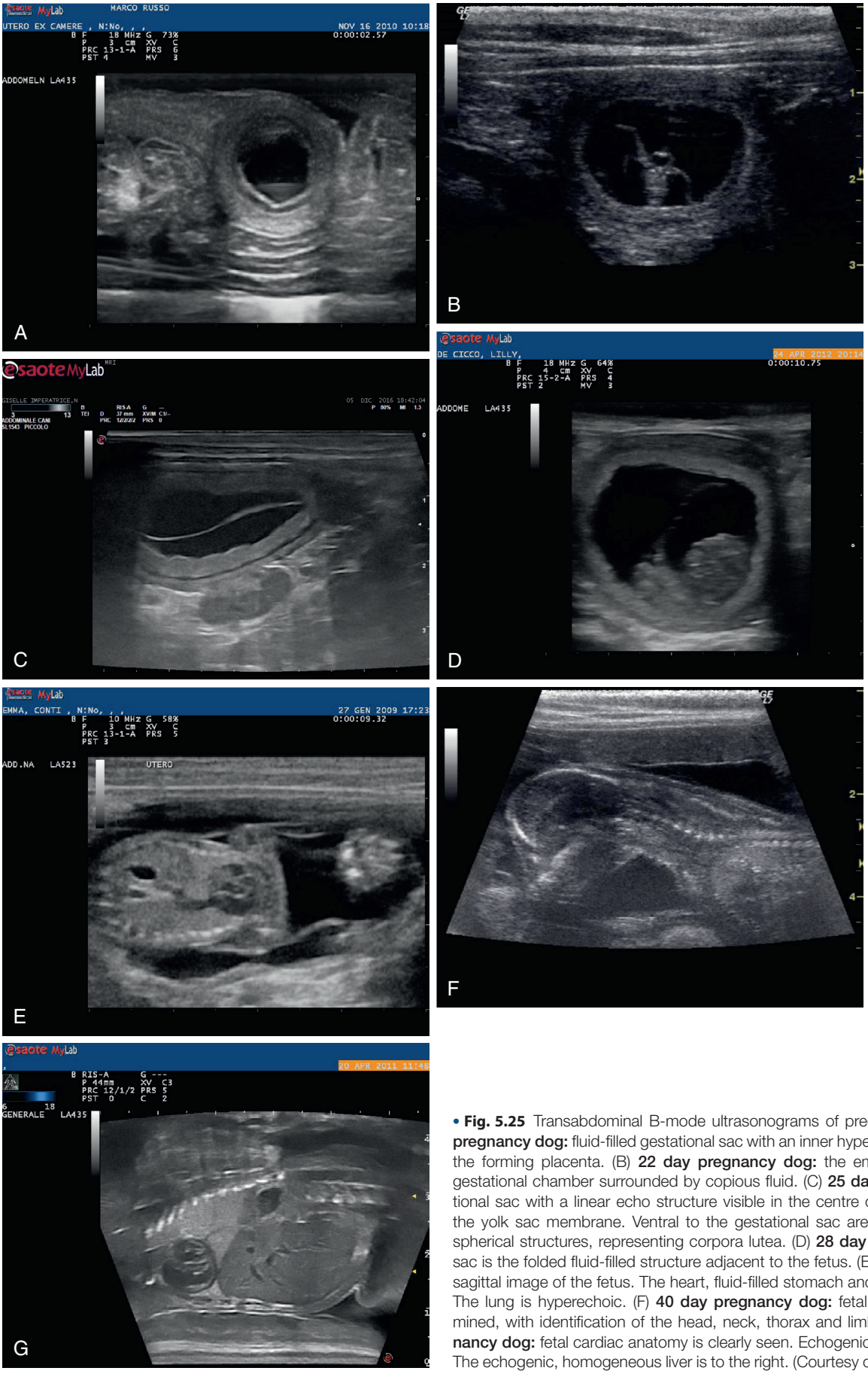

• **Fig. 5.25** Transabdominal B-mode ultrasonograms of pregnant bitches. (A) **20 day pregnancy dog:** fluid-filled gestational sac with an inner hyperechoic band representing the forming placenta. (B) **22 day pregnancy dog:** the embryo is visible within the gestational chamber surrounded by copious fluid. (C) **25 day pregnancy dog:** gestational sac with a linear echo structure visible in the centre of the image, representing the yolk sac membrane. Ventral to the gestational sac are visible three hypoechoic, spherical structures, representing corpora lutea. (D) **28 day pregnancy dog:** the yolk sac is the folded fluid-filled structure adjacent to the fetus. (E) **35 day pregnancy dog:** sagittal image of the fetus. The heart, fluid-filled stomach and liver are easily visualised. The lung is hyperechoic. (F) **40 day pregnancy dog:** fetal orientation can be determined, with identification of the head, neck, thorax and limb buds. (G) **50 day pregnancy dog:** fetal cardiac anatomy is clearly seen. Echogenic lung surrounds the heart. The echogenic, homogeneous liver is to the right. (Courtesy of Professor Marco Russo.)

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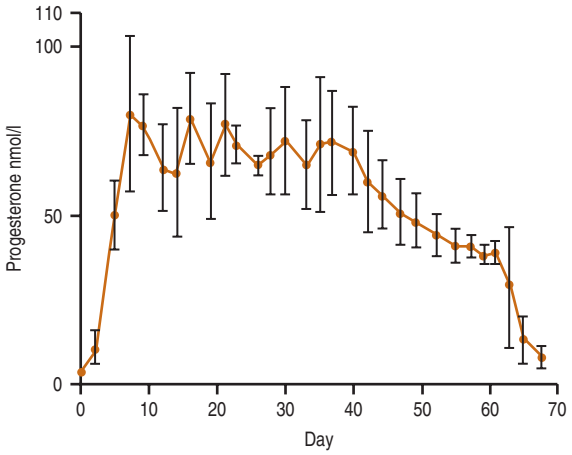

• **Fig. 5.26** Mean (+/- SD) plasma concentrations of progesterone throughout pregnancy (four cats). Day 0 represents the day of mating. (Courtesy Prof. T. J. Gruffydd-Jones.)

The average gestation length is 63 to 65 days (Prescott 1973) but is reported to vary between 59 and 70 days. Kittens born after a gestation length shorter than 59 days generally show decreased viability. The fact that ovulation may not necessarily occur after the first mating may partly explain the considerable variation in gestation length.

The modal litter size for nonpedigree cats is four, with a range of two to seven, but there is variation in litter size between pedigree breeds. Oriental breeds tend to have larger litters, sometimes in excess of 10 kittens, whereas pedigree longhair queens tend to have smaller litters, often of only two or three kittens. Singleton litters are unusual, which may reflect fetal resorption due to inadequate fetoplacental endocrine contribution to the maintenance of pregnancy.

A proportion of queens will display oestrous behaviour while pregnant. Some of these cats will mate, and this could lead to superfetation (see Chapter 9), although this has not been convincingly proved (Root Kustritz 2006a). Free-living queens may mate with several competing toms during oestrus, and hence superfecundity is common (see Chapter 9).

Methods of Pregnancy Diagnosis

Cats lend themselves particularly well to pregnancy diagnosis by abdominal palpation. This is most satisfactorily performed 16 to 26 days after mating, when conceptuses are readily identifiable as individual, turgid, spherical swellings. Pregnancy may be confirmed as early as 13 days after mating, but at this stage the conceptuses may be confused with faecal boluses. After 6 weeks the conceptual swellings increase markedly in size, elongating and merging, thus making palpation more difficult. However, by this stage there will usually be significant abdominal enlargement. Radiography has also been used for pregnancy diagnosis and for an accurate prediction of parturition date in cats (Haney et al. 2003).

The first report on the use of ultrasonography in pregnant cats was published in 1980 (Mailhac et al. 1980). Detailed descriptions of B-mode ultrasonographic images of the pregnant feline uterus have been reported for the first (Zambelli et al. 2002) and second half of gestation (Zambelli et al. 2004). The earliest ultrasonographic observation of a gestational sac was on day 10 after mating (with a 10 MHz transducer), whereas the embryo proper could be visualised from day 18 onwards (Fig. 5.28). Davidson et al. (1986) were the first to report the use of ultrasonography for pregnancy diagnosis. Fetal cardiac activity can be detected from day 16 to 17 onwards to assess fetal viability (Root Kustritz 2006b). In a recent study (De Haas van Dorsser et al. 2006) urinary relaxin levels in domestic and nondomestic felids appeared to mirror the profiles in plasma, and measurements of relaxin in urine therefore provide a sensitive and specific biochemical method for pregnancy diagnosis in cats. 'Bench-top kits' have been developed to detect relaxin in urinary samples of both domestic and wild felids for a rapid pregnancy test (De Haas van Dorsser et al. 2007, Harris et al. 2008). Measurements of prostaglandin-F metabolite (PGFM) levels in faecal samples of domestic and several wild cat species also appear promising as a noninvasive test for pregnancy (Dehnhard et al. 2011).

Prevention and Termination of Pregnancy

In all domestic species there will be occasions when it will be desirable to either prevent pregnancy from occurring or terminate it prematurely. Such occasions may follow an unintended mating (misalliance), in which pregnancy and parturition may present a severe risk to the dam's health or in which the owners of the animal do not want the pregnancy to continue.

Horse

The management and treatment of twinning are discussed in detail in Chapter 31. If the pregnancy needs to be curtailed for this and other reasons, such as mismating, the treatment of choice is $PGF_{2\alpha}$ or an analogue administered after the CL has become responsive to the hormone, i.e., 4 days after ovulation (see Chapters 1 and 5) and before the formation of the endometrial cups, i.e., about 35 days. Therefore it is preferable to treat approximately 10 to 15 days after mating. Alternatively, intrauterine infusion of 250 to 500 ml of physiological saline during the same period will also be effective because, as well as a physical effect in flushing out the conceptus, it also stimulates the release of endogenous $PGF_{2\alpha}$.

Cattle

Pregnancy can be terminated from 4 to 100 days after ovulation with $PGF_{2\alpha}$ or an analogue, and even up to 150 days many cows will respond. Subcutaneous injections of a progesterone-receptor antagonist at days 46 and 47 appeared also effective as an abortive treatment in cattle (Breukelman et al. 2005b). After 150 days the placenta forms an additional source of progesterone for the maintenance of pregnancy, until about 270 days of gestation; this implies that in many cases the use of $PGF_{2\alpha}$ alone will not be effective. During this period long-acting corticosteroids either alone or in combination with $PGF_{2\alpha}$ are required (see Chapter 6).

Goat

Pregnancy can be terminated at any stage with a luteolytic dose of $PGF_{2\alpha}$ (Day and Southwell, 1979).

Sheep

$PGF_{2\alpha}$ is effective in terminating pregnancy after day 4 and before day 12. During the days 12 to 21 period there will be no response because of the protective effect of oIFN-tau, which ensures the

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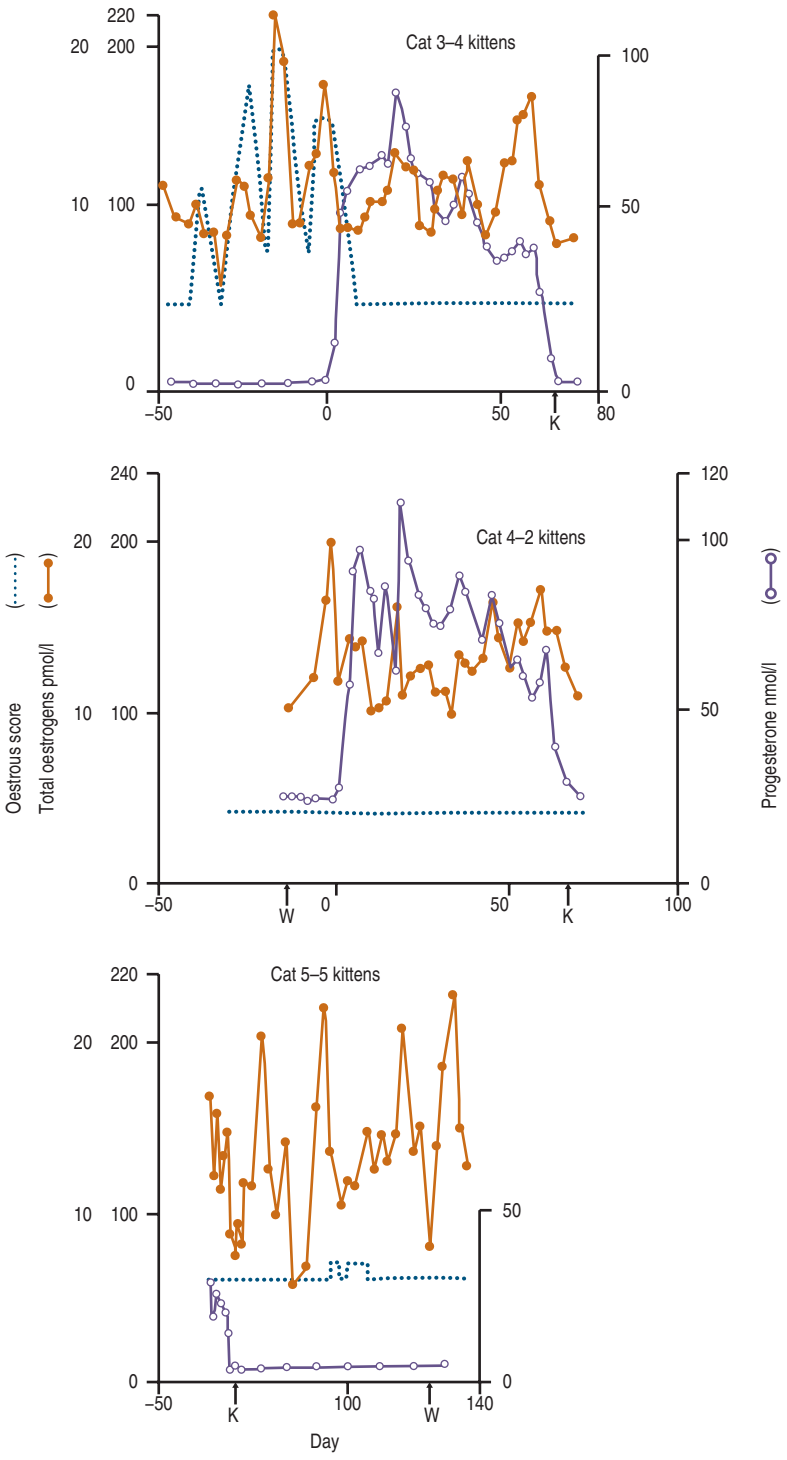

• **Fig. 5.27** Plasma concentrations of total oestrogens (○——○) and progesterone (●——●), and oestrous scores (– – – – –) throughout oestrus, pregnancy and lactation in individual queens. Day 0 represents the day of mating; *K,* The day of kittening; *W,* the day of weaning. (Courtesy of Prof. T. J. Gruffydd-Jones.)

survival of the CL. There is also some suggestion of another refractory period between days 25 and 40. After 45 to 55 days the CL is no longer the main source of progesterone for the maintenance of pregnancy, and the placenta starts to be the dominant production site. During the third and fourth month of gestation, induction of abortion is more difficult, although the use of the progesterone-receptor antagonist aglepristone appears to be effective in some ewes at this stage (Taverne et al. 2006). During the final weeks of gestation, corticosteroids will be necessary to terminate pregnancy (see Chapter 6).

Pig

Pregnancy can be terminated at any stage with $PGF_{2\alpha}$, although repeated injections are sometimes necessary to accomplish expulsion of all conceptuses.

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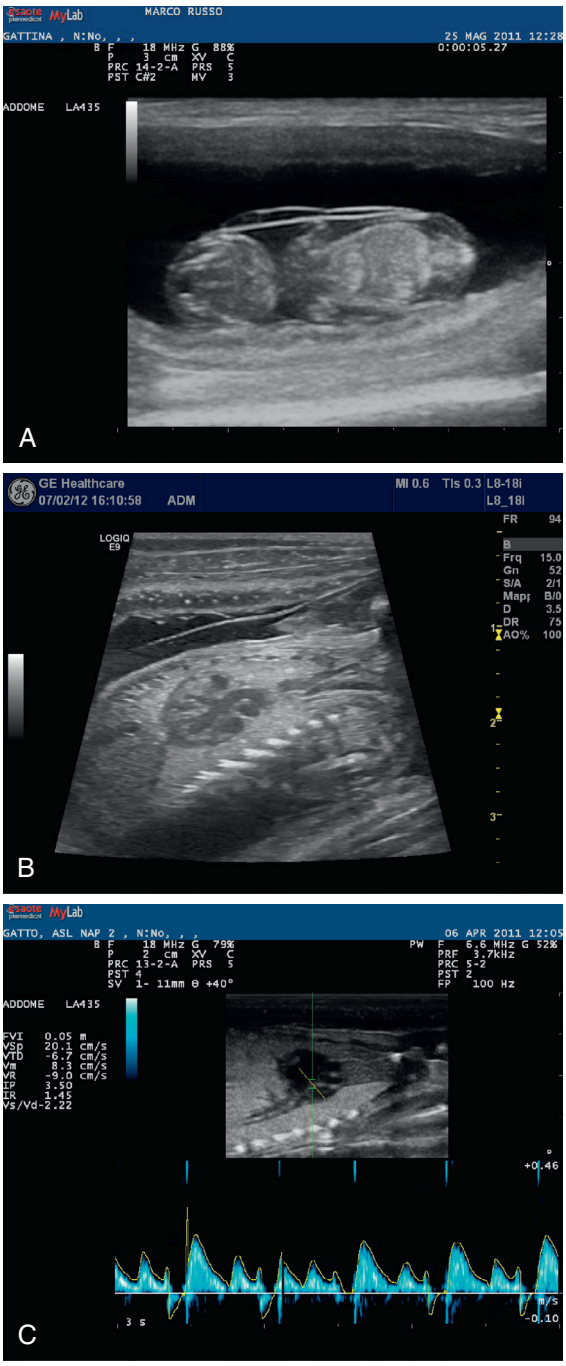

• **Fig. 5.28** Transabdominal B-mode ultrasonograms of pregnant queen cats. (A) **30 day pregnancy cat:** sagittal image of the fetus. Head, thorax and abdomen are clearly visible. (B) **42 day pregnancy cat:** sagittal image of the fetal thorax. Cardiac chambers and large vessels are clearly visible. The surrounding lung is hyperechoic. Hyperechoic ribs with a distinct distal shadowing are visible. (C) **45 day pregnancy cat:** longitudinal view of the fetal thorax with flow velocity waveforms from the heart. (Courtesy of Professor Marco Russo.)

Dog

Pregnancy can be prevented in the bitch by the strategic use of oestrogens during the first 5 days after mating. They exert their effect by interfering with the transport of the zygotes from the uterine tube to the uterine horns, probably by causing oedema of the endosalpinx and thus a temporary tubal occlusion. For many years estradiol benzoate was used as a single intramuscular or subcutaneous injection at a dose rate of 5 to 10 mg. More recently, the use of lower doses of estradiol benzoate 0.01 mg/kg administered on the third, fifth and possibly seventh days after mating has been advocated because it is possibly a safer option.

Attention has also focused on the termination of pregnancy at a later stage. Natural $PGF_{2\alpha}$ rather than analogues has been found to be effective when administered at a dose rate of 150 to 270 mug/kg subcutaneously twice daily consecutively on days 10 to 14 after the bitch has entered metoestrus or pregnancy, as confirmed by exfoliative vaginal cytology (see Chapter 1) (Romagnoli et al. 1993). Earlier reports, in which pregnancy was terminated at a later stage, namely 25 to 30 days, resulted in unacceptable side effects (Lein et al. 1989). At present it is doubtful if $PGF_{2\alpha}$ should be used for this purpose. The use of progesterone-receptor antagonists appeared a more effective and acceptable treatment (Concannon et al. 1990, Linde-Forsberg et al. 1992, Galac et al. 2000, Fieni et al. 2001). Abortion can be induced not only by blocking the effect of progesterone at the level of its receptors but also by inhibiting its synthesis by the CLs with an antiluteotrophic therapy.

Dopamine agonists, such as cabergoline that inhibit prolactin secretion and hence indirectly withdraws the luteotrophic support for the CLs, have been used to terminate pregnancy in bitches. It has been used at a dose rate of 1.65 mug/kg subcutaneously for 5 days at 25 to 40 days of gestation (Onclin et al. 1993). Unlike the prolactin inhibitor bromocriptine, it does not have unpleasant side effects but, because a resultant uterine inertia (see Chapter 13), may result in retention of puppies that become macerated (see Chapter 9), it is not without dangers. Most commonly, cabergoline is administered orally daily (5 mug/kg), whereas cloprostenol (5 mug/kg) is given by other parenteral routes on alternate days. Abortion/resorption usually follows within 10 days after the start of treatment. The use of the above mentioned therapeutics for pregnancy termination has recently been reviewed extensively by Lamm and Makloski (2012). Termination of an unwanted pregnancy in the bitch should always be closely monitored, particularly with sequential transabdominal B-mode ultrasonography (Taverne et al. 1989).

Cat

Estradiol cypionate, by intramuscular injection at a dose rate of 125 to 250 mug within 40 hours of mating, has been shown to be effective in preventing pregnancy, probably by interfering with the normal transport of the zygotes within the uterine tubes (Herron & Sis 1974). Similarly, injections of diethylstilbestrol have been used. However, there are few data on possible side effects, and such treatments should only be used in exceptional circumstances. A single 5 mg dose of megestrol acetate is used by some within 1 day of mating.

Both $PGF_{2\alpha}$ and the dopamine agonist cabergoline have been shown to be fairly effective in causing abortion; however, the former has unpleasant side effects (Verstegen et al. 1993b). Regimens using cabergoline and low-dose cloprostenol appear to be most promising (Onclin & Verstegen 1997). More recently, aglepristone was shown to be effective in midpregnancy in a controlled clinical study with 66 queens with known breeding dates (Fieni et al. 2006). Two subcutaneous injections, given 24 hours apart, resulted in expulsion of all fetuses in 88.5 % of the animals within 14 days after the first injection. The use of the above mentioned therapeutics

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for pregnancy termination in cats has been reviewed extensively by Lamm and Makloski (2012).

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6

Parturition and the Care of Parturient Animals and the Newborn

MARCEL TAVERNE AND DAVID E. NOAKES

Parturition

It is essential for the veterinarian to be perfectly familiar with the normal course of parturition in domestic species in order to be able to differentiate between physiological and pathological birth. This implies that it is important to spend time observing normal parturitions in these species, although this cannot always be achieved easily. An appropriate intervention at the correct time can increase the likelihood of a successful outcome by ensuring that both mother and offspring survive.

Initiation of Parturition

Parturition is one of the most fascinating of biological processes, for although its physiology is explicable and its associated endocrine changes have been fairly well established, the factors which initiate birth and thereby terminate pregnancy after a constant length of gestation for a given species are still imperfectly understood. The modern concept, which is firmly based on experimental studies and clinical observations (mainly in farm animals and to some extent in horses) is that the fetus exerts an overriding control on the length of gestation and that the mother can influence the time of birth only within narrow limits.

The uterine musculature is the key component of labour, and the essential physiological changes between gestation and birth are a biochemical ripening of the cervix and liberation of the contractile potential of the myometrium; the factors involved in these transformations are humoral, biochemical, neural, and mechanical.

Of the humoral factors, the most important is the reversal of those mechanisms that are necessary for the maintenance of pregnancy—in particular the removal of the progesterone block, which ensures that, during this phase of the animal's reproductive life, the myometrium is largely quiescent. Together with the changes in the synthesis and secretion of relaxin, oestrogen, and prostaglandin, it also enhances the biochemical ripening of the connective tissues within the cervix in preparation for the dilatation of the latter.

The mechanisms that are responsible for the initiation of parturition vary slightly between species. Initially, much of the experimental work to determine them was done in the ewe because the sheep was used as a research model for parturition in women. This, together with circumstantial evidence obtained from cattle, sheep, and goats in which it was observed that prolonged gestation was usually associated with abnormalities of the fetal brain and adrenals, supported the hypothesis first advocated by Hippocrates that the fetus is responsible for controlling the time when parturition occurs.

The mechanisms are fairly well defined in the domestic ruminant species, but in the pig, horse, dog, and cat there are a number of important areas that are not well understood (Taverne & van der Weijden 2008). Because much of the work has been reported in sheep, it is proposed to describe in detail the mechanisms in this species and, subsequently, to indicate those differences that have been identified in other species.

Ewe

Parturition in the ewe occurs as result of activation of the fetal hypothalamus–pituitary–adrenal (HPA) axis; the areas of the hypothalamus involved in this process are the paraventricular nuclei. The fetal HPA axis is similar to the adult HPA axis, except that the fetal brain is still developing in late gestation, and the former communicates with the latter via the placenta. There is still uncertainty about the mechanisms responsible for the activation of the fetal hypothalamus. A number of theories have been proposed. These are:

- Maturation of the fetal hypothalamus which might result in the development of critical synapses in the paraventricular nucleus, allowing an increase in fetal neuroendocrine function;
- Ability of the hypothalamus to respond to the effects of placental hormones; and
- Fetal stressors such as hypoxia, hypercapnia, changes in blood pressure, and blood glucose (Wood 1999).

It is also postulated that placental-derived hormones, such as oestrogens, progesterone, PGE_2, or corticotrophin-releasing factor (CRF), may also act on the hypothalamus.

Let us consider the interactions between the pituitary and adrenal cortex. Before 120 days of gestation, much of the cortisol present in the circulation of the fetal lamb is derived from the ewe via transplacental transfer. During this period a possible fetal overexposure, for example in cases of maternal stress, is prevented by the action of the placental enzyme 11ß-hydroxysteroid dehydrogenase II that converts cortisol into cortisone. During the last 15 to 20 days of gestation, there is a progressive rise, in a semilogarithmic pattern in fetal cortisol concentrations, which reach a peak 2 to 3 days before birth, thereafter declining 7 to 10 days postpartum. The source of the increase in fetal cortisol is the fetal adrenal, which is due to both an increase in the size of the organ in relation to total body

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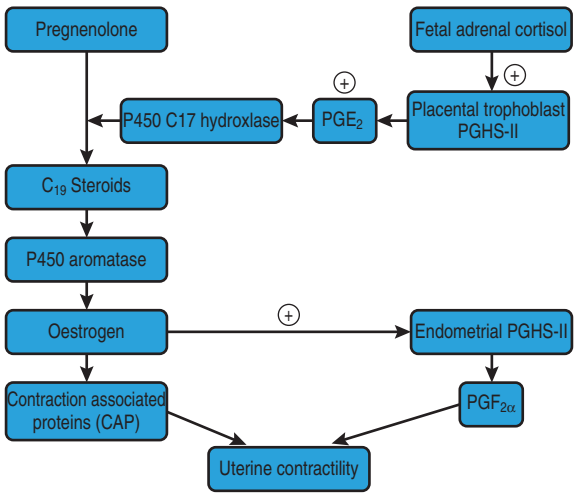
weight, and an increase in its sensitivity to adrenocorticotrophic hormone (ACTH), as a result of accelerated processing of ACTH from proopiomelanocortin (POMC); maternal cortisol concentrations only rise around the time of parturition (Wood 1999). At the same time the binding capacity of the fetal plasma increases, thus reducing the amount of free cortisol in the fetal circulation and thereby reducing the negative feedback effect on the secretion of ACTH by the fetal pituitary.

In sheep fetal pituitaries, the 'fetal' corticotrophs are replaced (probably accompanied by a change in the distribution of POMC peptides) by smaller stellate cells, the so-called 'adult' corticotrophs, around 125 days of gestation, which might reflect an increased potential for ACTH secretion (Antolovich et al. 1988). There is an increase in corticotrophin-releasing hormone (CRH) and arginine vasopressin (AVP) from the paraventricular and supraoptic nuclei in the fetal hypothalamus, and these are thought to be responsible for the initial (CRH) and late (AVP occurring in the last 10 days) rise in fetal plasma ACTH levels. Endogenous opioids may also play a role in stimulating ACTH secretion via their effect upon the fetal hypothalamus rather than the pituitary. It has been shown experimentally that when exogenous opioids are infused into the fetal lamb, there is an increase in ACTH which can be abolished by the administration of the opioid antagonist naloxone (Brooks & Challis 1988).

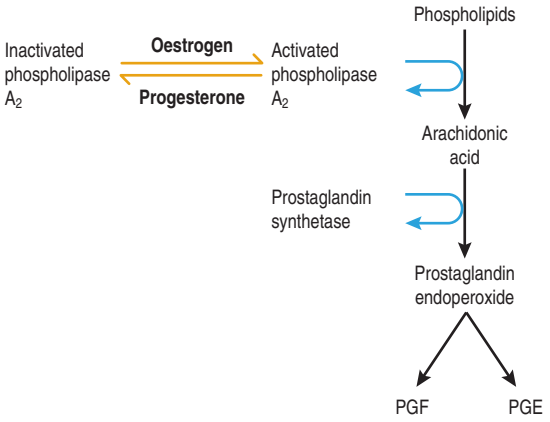
The fetal adrenal becomes more responsive to ACTH stimulation with advancing age (Glickman & Challis 1980). Maturation is induced by ACTH, particularly the pulse pattern of its secretion. It has been shown that insulin-like growth factors (IGFs) may have an autocrine and/or paracrine role in regulating ovine fetal adrenal function (Hann et al. 1992). Fetal growth hormone, which is elevated from 50 to 70 days of gestation and then falls until 100 days, before increasing to term, may also modify the response of the fetal adrenal to ACTH (Devaskar et al. 1981). There is recent evidence that maternal undernutrition around the time of conception, resulting in a loss of maternal body weight of some 15%, may programme the development of the fetal HPA axis in such a way that its activation (see later in this chapter) and the resulting birth of lambs take place some 7 to 10 days earlier (Kumarasamy et al. 2005).

An adapted version of the original hypothesis suggests that intrauterine prostaglandin synthesis is affected by this rise in cortisol by two different pathways (Whittle et al. 2000, 2001). The first, oestrogen-independent pathway, involves the enhanced expression of prostaglandin synthetase (PGHS-II) within the fetal trophoblast cells of the placenta. The resulting release of PGE2, as reflected by an early rise in fetal plasma E2 levels, stimulates the placental conversion of pregnenolone to C-19 steroids and their subsequent aromatisation to oestrogens in the placenta, by activating the enzymes 17α-hydroxylase and aromatase (Fig. 6.1). One of the consequences of the rise in oestrogens is a stimulation of PGHS-II in the maternal endometrium, leading to enhanced PGF production and release within the uterine wall; this will stimulate myometrial contractility. This represents the second, oestrogen-dependent pathway by which fetal cortisol affects PG production (Fig. 6.2). In ruminants such as the cow and goat, in which progesterone is predominantly produced by the corpus luteum, this PGF might also induce luteolysis (see later in this chapter).

Oestrogens themselves also have a direct effect upon the myometrium by increasing several so-called contraction-associated proteins (CAP's), such as connexins (to form gap junctions; these special areas of contact between smooth muscle cells, allow the passage of electrical pulses, thus ensuring coordinated contractions), oxytocin



• **Fig. 6.1** Model of endocrine events associated with the onset of ovine parturition. (From Whittle et al. 2000.)



• **Fig. 6.2** The induction of prostaglandin E (PGE) and prostaglandin F (PGF) release (after Liggins 1982).

and prostaglandin receptors, and calcium channels. Oestrogens also stimulate softening of the cervix by altering the structure of collagen fibres. Prostaglandins thus play a key role in initiating parturition and are considered to be the main intrinsic stimulating factor of smooth muscle cells (Csapo 1977); thus its release is crucial in initiating myometrial contractions. Prostaglandin synthase inhibitors have shown to be effective in delaying onset of parturition in a wide range of species, including sheep, pigs, and women (Rac et al. 2006, Taverne et al. 1982).

The effect of the parturient contractions of the myometrium is to force the fetal lamb towards the cervix and vagina where it will stimulate sensory receptors and initiate Ferguson's reflex, with the release of large amounts of oxytocin from the posterior pituitary. However, in ruminants this oxytocin release is mainly detected in peripheral plasma during the expulsive stage of parturition (Hydbring et al. 1999), i.e., many hours after the switch from a gestational to a parturient-type of myometrial activity has already taken place. At this rather late stage of parturition, oxytocin will further stimulate myometrial contractions and enhance release of $PGF_{2\alpha}$ from the myometrium, and this positive feedback system of increasing magnitude thus supports a rapid expulsion of the fetus.

Other important changes which are brought about by the endocrine events of parturition have been observed. For instance,

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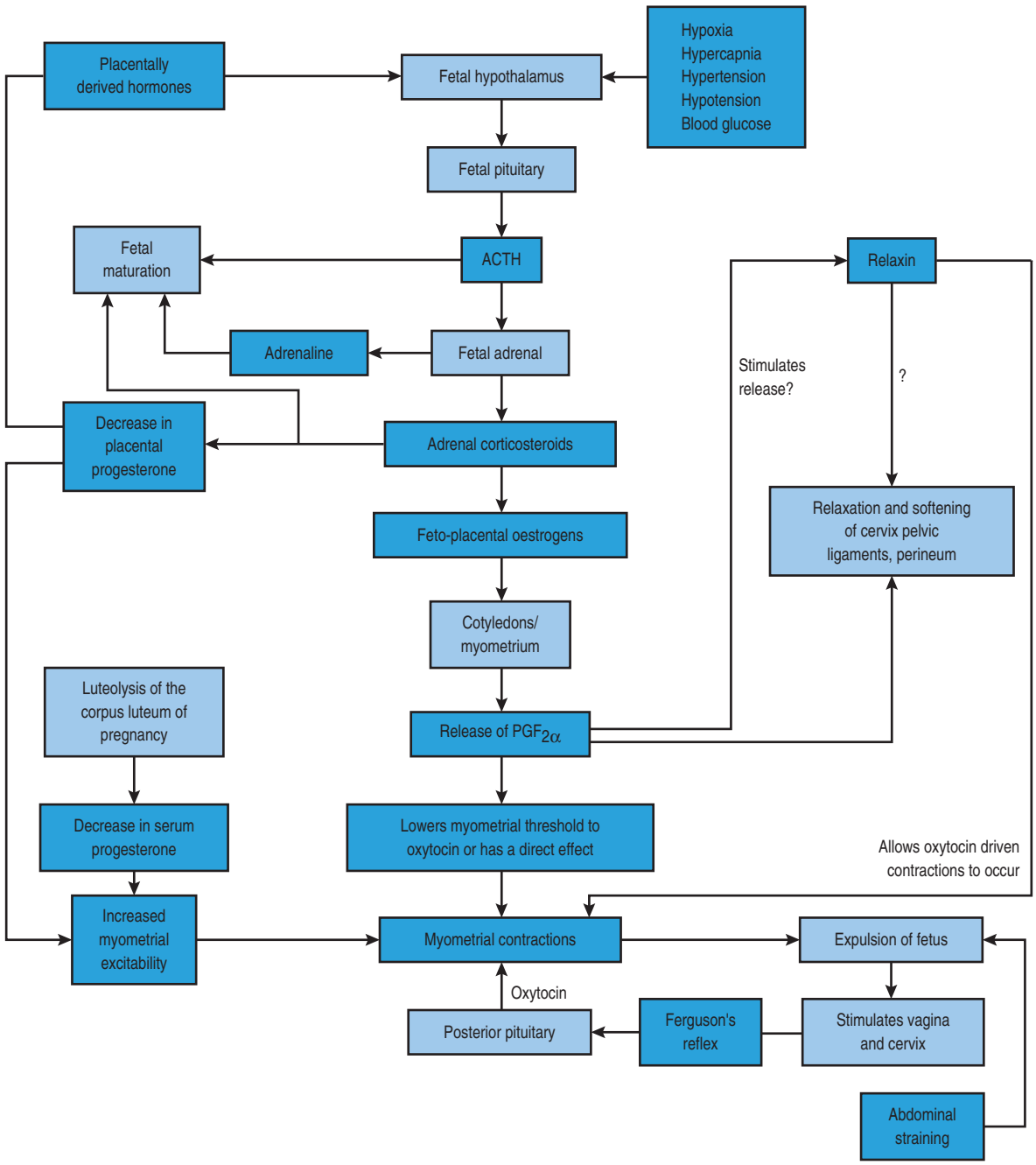

• **Fig. 6.3** The endocrine changes that occur before and during parturition in the sow, ewe, and cow and their effects.

maturation of the fetal lamb's lungs, especially the production of alveolar surfactant, is stimulated by cortisol, as are many other changes in fetal function and structure that enable the lamb to survive after birth (Fowden 1995, Fowden et al. 2016).

A schematic, representation of the endocrine changes that are involved in the initiation of parturition in the ewe and some other species is shown in Fig. 6.3. Overall trends in reproductive and other hormones occurring in the peripheral circulation of the ewe around the time of parturition are shown in Fig. 6.4.

Cow

The corpus luteum is the main source of progesterone throughout bovine pregnancy, but as stated in Chapter 5, the placenta assumes a role of progesterone production between about 150 and 200 days of gestation. So if the ovary containing the corpus luteum (CL) or the CL itself is removed during this stage, pregnancy may continue in some or all cases (McDonald et al. 1953, Lindell 1981). Although the CL is not required to maintain pregnancy at all stages of gestation, it has been shown that its regression plays an important role in the endocrine changes that are necessary for the initiation of parturition. There is no evidence to suggest that the prepartum decline of maternal plasma progesterone levels is due to withdrawal of a maternal luteotrophic support. Although other mechanisms, such as a direct effect of glucocorticoids, may be involved, it is most likely that luteal regression is initiated by the action of $PGF_{2\alpha}$. The prepartal rise in fetal cortisol stimulates

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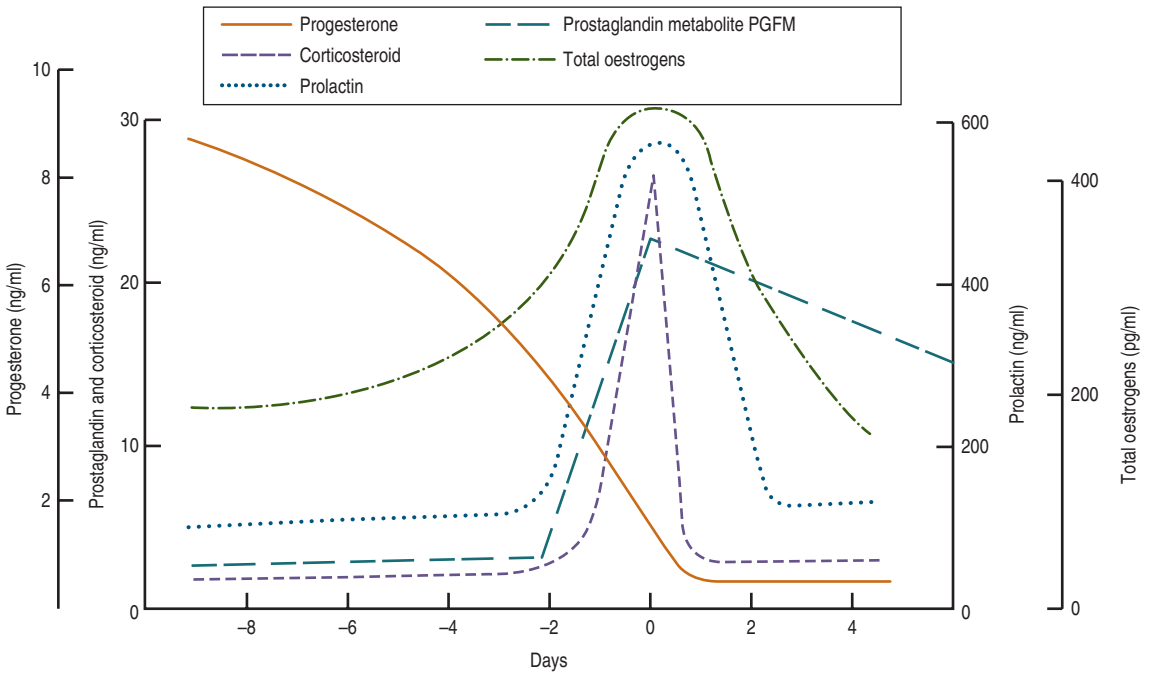

• **Fig. 6.4** Trends in hormone concentrations in the peripheral plasma of the ewe around the time of parturition. Day 0 at parturition.

the expression of the enzymes 17α-hydroxylase-C17 (CYP17) and cyclooxygenase 2 (COX2) in the uninucleate trophoblast cells of the bovine placenta (Schuler et al. 2006a, b). These enzymes play a key role in the synthesis of oestrogens and prostaglandins by the placenta. Yet the release of prostaglandins into the maternal circulation, as mirrored by its main metabolite, shows only a gradual prepartum increase during the rapid and final prepartum decline of plasma progesterone. However, unlike during the oestrous cycle, its amplitude is relatively small and does not show clear pulses (Königsson et al. 2001). Apart from the (fetal) placenta, the bovine uterus itself appears to be able to produce prostaglandins, and this becomes even clearer during the postpartum period (see later in this chapter). The endocrine changes responsible for initiating parturition are rather similar, but not identical, to those described in the sheep (see Fig. 6.3 and Shenevai et al. 2012), on which much of the experimental work has been done. In fact, the first reports on a possible involvement of the fetal hypophyseal–adrenal axis in the initiation of parturition came from pathological observations in cows with a hereditary form of prolonged gestation. Fetuses from these animals showed aplasia of the pituitary and adrenal hypoplasia (Kendrick et al. 1957).

The overall trends in reproductive and other hormones occurring in the peripheral circulation of the cow around the time of parturition are shown in Fig. 6.5.

Doe (Nanny) Goat

In this species the CLs provide the essential source of progesterone necessary for the maintenance of gestation because ovariectomy or extirpation of the CLs will terminate pregnancy (Irving et al. 1972). There is a positive association between fetal plasma ACTH and the prepartal increase of fetal cortisol levels (Ford et al. 1998). Placental 17α-hydroxylase, which is stimulated by the rise in fetal cortisol (Flint et al. 1978), diverts the synthesis of progesterone by the CLs into oestrogen. The change in the oestrogen to progesterone ratio stimulates $PGF_{2\alpha}$ synthesis as in the ewe (see Figs 6.1 and 6.2), resulting in luteolysis with a further decline in progesterone. Progesterone disappears from the circulation before parturition can occur. Yet this sequence of events has been disputed by Ford and associates (1998), who found no clear evidence that prepartum luteolysis in goats is associated with changes in the basal concentrations of PG's in maternal arterial or uterine–ovarian–venous plasma.

Sow

In the sow, progesterone from the CLs is necessary for the maintenance of pregnancy throughout its entire duration. Fetal participation in the initiation of farrowing is indicated by the prolongation of pregnancy after fetal decapitation and hypophysectomy. If only one fetus survives to term in a litter, this fetus appears capable of initiating the farrowing process, but in the presence of four or more decapitated litter mates, a single intact fetus is incapable of doing so (Stryker & Dziuk 1975). Furthermore, when fetal plasma cortisol is raised prematurely in all or part of a (reduced) litter, then farrowing is not induced (Randall et al. 1990). These observations indicate that the mechanisms of fetal participation in the initiation of farrowing are far from clear at the moment. Parturition is preceded initially by increased levels of cortisol in the fetal plasma, although the pattern of fetal plasma cortisol concentrations is quite different in individual piglets, even when fetuses within one litter are compared (Randall 1983). It is assumed that the rise in fetal cortisol results in a rise in the maternal blood oestradiol and $PGF_{2\alpha}$ metabolites and a decrease in progesterone. Yet on the day of farrowing, progesterone levels are well higher than baseline, and the decrease continues during the first 24 hours after parturition (Peltoniemi & Oliviero 2011). Although the prepartum decline of plasma progesterone coincides with the first increase of $PGF_{2\alpha}$ metabolites according to some authors (Kindahl et al. 1982), others found no such clear relationship (Silver et al. 1979, Randall et al. 1986). It is unlikely that oestrogens are responsible for stimulating the release of $PGF_{2\alpha}$ as occurs in the other species

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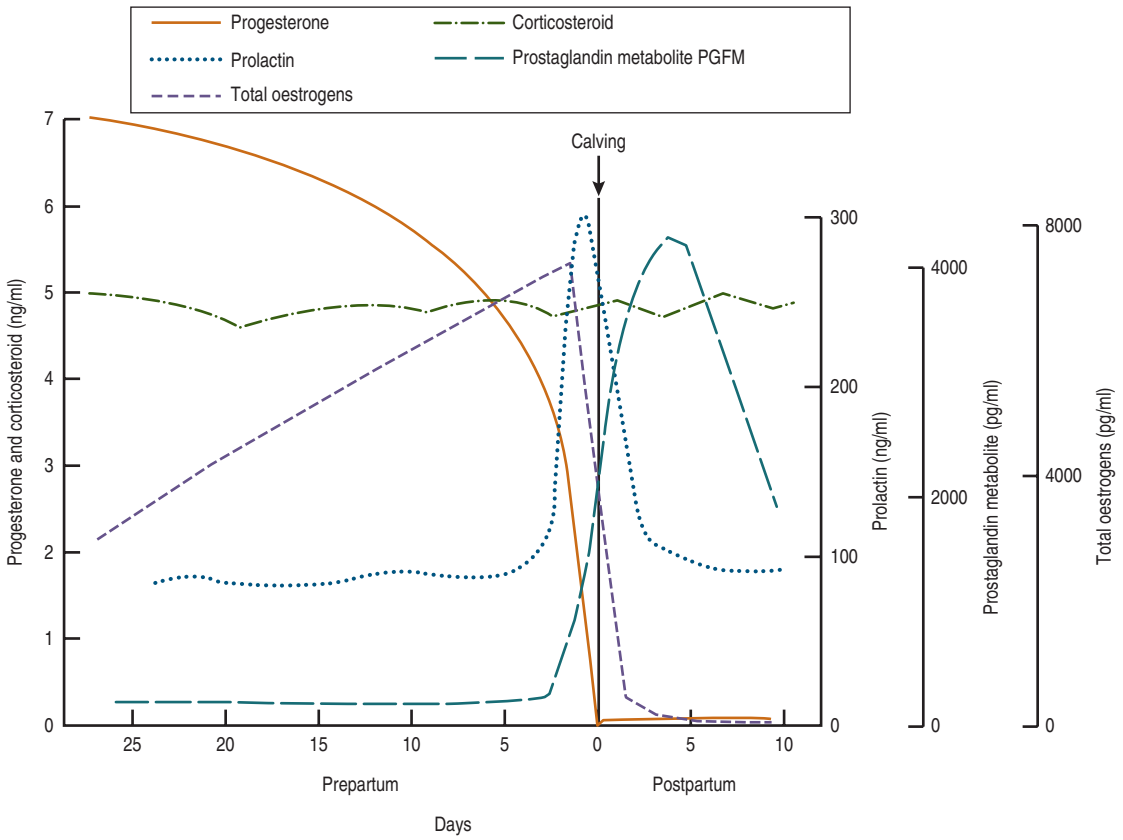

• **Fig. 6.5** Trends in hormone concentrations in the peripheral plasma of the cow around the time of parturition. Day 0 at parturition.

(First 1979), and exogenous oestrogens will not induce farrowing. The hormonal changes in the maternal peripheral circulation are illustrated in Fig. 6.6.

Mare

The mechanisms responsible for the initiation of parturition are not yet as well understood as those of the ruminant species. However, it is likely that the fetal foal is responsible for the initial trigger mechanism because the fetal adrenal undergoes rapid hypertrophy immediately before parturition (Comline & Silver 1971), and fetal plasma cortisol concentrations have been shown to increase nearly 10-fold during the last days before foaling (Card & Hillman 1993, Cudd et al. 1995). The main differences from the ewe relate to a much wider range of gestational length (Davies Morel et al. 2002) and the timing and magnitude of key endocrine changes that occur in the maternal circulation (see Fig. 5.1). When the secondary corpora lutea on the ovaries stop producing progesterone, maternal progesterone reach very low levels during the last third part of pregnancy, and it appears that during this stage metabolites of both progesterone and pregnenolone, collectively named progestagens, are responsible for myometrial quiescence (Ousey 2004, Fowden et al. 2008). In fact, during the last 20 to 30 days of gestation, total progestagen levels increase and reach a peak about 48 hours before parturition. It is only very close to the time of delivery (during the last 24–48 hours) that these levels show a steep decline (Conley 2016). These changes in progestagen levels appear to be under the control of the fetal HPA axis.

When the fetal adrenal cortex grows and differentiates, the fetal adrenals start to secrete pregnenolone that serves as a precursor for progestagens synthesis by the uteroplacental tissues. This explains why maternal plasma progestagens decline immediately upon intrauterine death of the fetus. When the adrenal cortex becomes further differentiated and activated by the prenatal rise of fetal ACTH, it switches from pregnenolone to cortisol production, which appears to be responsible for the final precipitous drop in maternal plasma progestagen levels (Fowden et al. 2008). Plasma oestrogen concentrations decline during the last 100 days of gestation rather than increase as in other species, reaching relatively low levels at parturition. However, this is largely a reflection of the decline in oestrone and the species-specific oestrogens, equilin and equilenin, because concentrations of oestradiol–17ß remain fairly constant. However, during the final 24 to 48 hours before delivery, maternal oestradiol–17ß concentrations double again (Barnes et al. 1975). Maternal prostaglandin levels are low during the major part of gestation, rising only slightly towards term, and then show a steep increase only at parturition (Vivrette et al. 2000, Davies Morel 2008, Fowden et al. 2008); this is probably because synthesis of primary PG's is stimulated whereas their conversion into metabolites is inhibited by the influence of the very rapid change of the progestagen/oestrogen balance.

Bitch and Queen Cat

Even less is known about the mechanisms that are responsible for initiating and controlling parturition in either of these two species. There are no published data on fetal cortisol secretion during late gestation. In the bitch a prepartum rise of cortisol has been detected in the peripheral circulation, with peaks obtained 8 to 24 hours prepartum (Concannon et al. 1975), but this increase is believed

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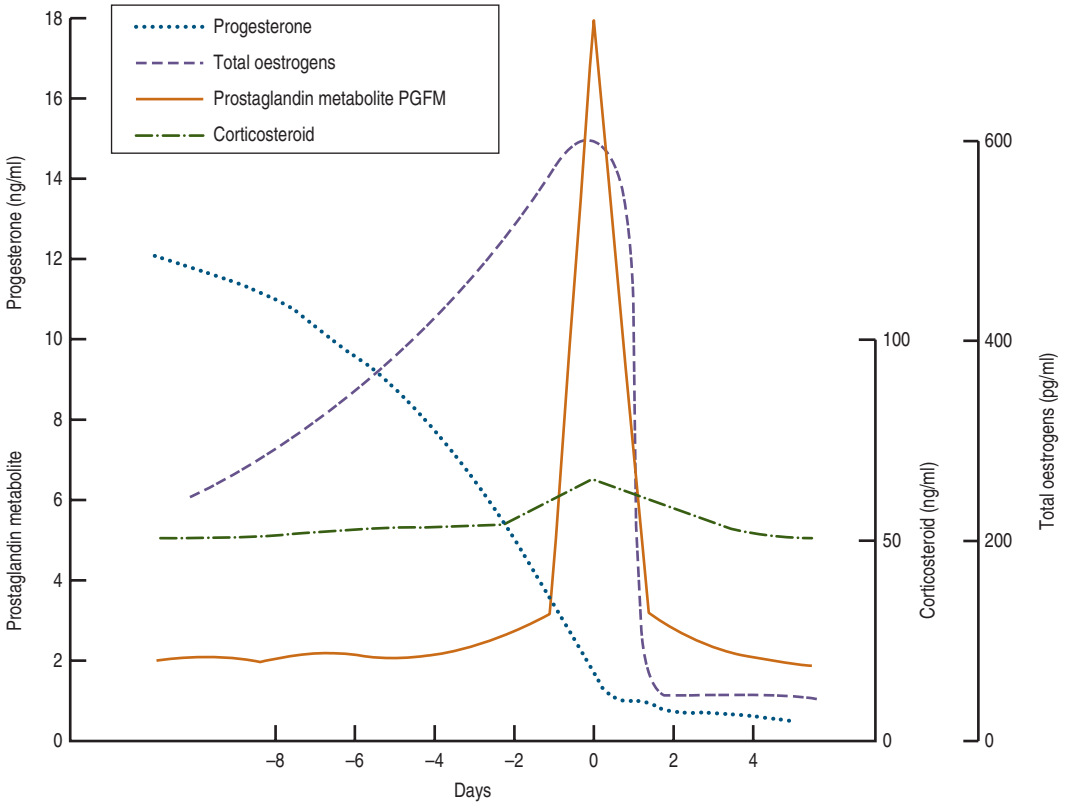

• **Fig. 6.6** Trends in hormone concentrations in the peripheral circulation of the sow around the time of parturition. Farrowing at day 0.

to reflect the stress associated with nest building, physical activity, and the onset of uterine contractions. Progesterone concentrations start to decline gradually from about the 30th day of gestation, and there is a precipitous fall between 12 and 40 hours before the birth of the first puppy (Concannon et al. 1975, Baan et al. 2008). This latter change coincides with an increased release of $PGF_{2\alpha}$ because, during the 48 hours before whelping, there is a rise in the metabolite PGFM. This prostaglandin production appears to originate from fetal trophoblast cells in the placentas; expression of the PG-synthesis regulating enzyme COX-2 is strongly upregulated in these cells during the immediate prepartum period (Kowalewski et al. 2010). It is tempting to speculate that this PG release causes luteolysis, but there is also evidence that other intraluteal processes, such as invasion of macrophages, massive expression of apoptotic enzymes, and significantly decreased expression of the steroidogenic regulatory, are involved in completion of luteolysis when the influence of progesterone is declining (Kowalewski 2014). Maternal plasma oestrogens remain at a fairly constant and very low level throughout pregnancy and start to decline about 2 days prepartum, reaching nonpregnant values at the time of parturition (see Fig. 5.21). Prolactin increases as progesterone concentrations decrease 1 to 2 days before whelping (Concannon et al. 1977, Baan et al. 2008); it is not known if this hormone plays any role in parturition. The few published data on maternal oxytocin levels indicate that oxytocin is not responsible for the initiation of whelping because levels were found to be elevated only once the expulsive stage had started (Olsson et al. 2003, Klarenbeek et al. 2007).

In the queen cat, progesterone remains at between 20 and 50 ng/mL for the first two-thirds of gestation before starting to decline gradually towards term. Just before parturition it declines more rapidly from 4 to 5 ng/mL to levels lower than 2 ng/mL at the time of parturition (Verhage et al. 1976, Schmidt et al. 1983). Prostaglandins levels in plasma are still unexplored in cats, but in pregnant wild and domestic cats, faecal PG levels were found to be elevated during pregnancy, peaking towards parturition (Dehnhardt et al. 2012). Oestradiol concentrations reach peak levels about 1 week before parturition and have declined substantially on the day of parturition.

The Role of Relaxin

Sources of Relaxin

Relaxin, a polypeptide hormone, belongs to the insulin superfamily of which the peptides consist of two chains connected by disulfide bridges. Except for humans and higher primates, in which three peptides are present in the genome, most mammals have two genes of which the RLN1 gene is the functional one (Klein 2016). Relaxin was shown to be responsible for causing relaxation of the pubic symphysis of guinea pigs by Hisaw in 1926. The most potent sources of this hormone are the CLs of the pregnant sow; however, it is now known to be produced by a number of other tissues such that it has a wide diversity of chemical structure and physiological effects between species (Bathgate et al. 2006, Klein 2016).

In the pig, as well as being present in the CLs of the pregnant sow, it is also produced by preovulatory follicles. Plasma levels are low, but physiological, during the final weeks of gestation and peak shortly before the onset of farrowing at the time of the prepartum decline of progesterone (Sherwood et al. 1981, Taverne et al. 1982). In the horse, dog, and cat the main or sole source of the hormone is the placenta. In horses the noninvasive trophoblast of the early equine embryo expresses relaxin (Klonisch et al. 1997). In

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a pregnant mare's plasma, concentrations start to rise from about 80 days of gestation and peak during parturition, although there is considerable breed variation (Stewart et al. 1992). In the bitch relaxin increases from about 4 weeks of gestation and remains elevated until term, whereas in the cat there is a sudden rise from 23 days of gestation with a peak at 36 days and a dramatic decline just before parturition (Stewart & Stabenfeldt 1985). The RLN1 gene has not been discovered in ruminants, and a relaxin peptide has not been isolated (Bathgate et al. 2006). Yet in the cow the CL appears to be the main source of a relaxin-like hormone (insulin-like peptide 3; INSL3), thus early studies, reporting that relaxin values increase just before calving (although still at relatively low concentrations), have to be interpreted with caution because it is not clear what substance was measured by the assay. In the ewe there is also conflicting data concerning the presence and secretion of a relaxin hormone, and it appears possible that in sheep, INSL3 substitutes for relaxin (Bathgate et al. 2006).

Actions of Relaxin

Crude extracts and purified forms of the hormone have a wide range of actions on a variety of target tissues, including the pubic symphysis, pelvic ligaments, cervix, myometrium, and the mammary gland (Bathgate et al. 2006).

In the sow relaxin stimulates the growth of the vagina, cervix, uterus, and mammary gland during late pregnancy (Min et al. 1997) and causes softening of the cervix before parturition, as judged by the ability to pass a series of rods of different diameters into the cervical canal during the last 3 days before farrowing (Kertiles & Anderson 1979). The latter changes, which are also influenced by the oestrogen to progesterone ratio, involve changes at a biochemical level by influencing the glycosaminoglycans to collagen ratio (O'Day-Bowman et al. 1991) and histological structure (Winn et al. 1993).

Despite the fact that there is not a reliable assay for relaxin in the cow and that the cow only produces a relaxin-like protein, there is circumstantial evidence that relaxin can influence cervical relaxation at term. Studies have shown that when highly purified porcine relaxin was placed directly on the external os of the cervix at 276 to 278 days of gestation, cervical relaxation occurred 8 to 12 hours later (Musah et al. 1986). Similar results have been obtained when parturition has been induced with dexamethasone (Musah et al. 1987). The reports on the effect of porcine relaxin on the cervix of the sheep are equivocal. Studies by Roche and associates (1993) has shown that a relaxin-like mRNA cannot encode a functional relaxin molecule, which suggests that sheep may not produce relaxin, and thus in this species, cervical relaxation may not be relaxin-dependent.

Relaxin also exerts an influence on myometrial activity, with several studies reported in domestic species, in particular the pig. In general, relaxin reduces both the frequency and amplitude of uterine contractions, particularly the former. It appears to act in concert with progesterone, oestrogens, oxytocin, and prostaglandins. Thus in the sow, although the progesterone concentrations have fallen significantly 10 to 24 hours before farrowing, with the removal of the progesterone block (see later in this chapter), myometrial activity is low (Taverne et al. 1979a). At this time, relaxin concentrations increase significantly. Furthermore, in sows there is a relationship between relaxin concentrations and the duration of farrowing (Wathes et al. 1989). After farrowing relaxin is present in the milk of the sow, and uptake during the first 48 hours may affect developmental programming of uterine and other somatic tissues of the newborn piglets (Yan et al. 2006).

Fetal Maturation

As has already been described, the fetal endocrine changes that occur in late pregnancy not only initiate parturition, but also stimulate a variety of maturational changes that enable the newborn animal to survive (Silver 1990, Mellor 1993). In the absence of these changes, sometimes referred to as 'preparation or readiness for birth' (Liggins et al. 1979, Rossdale & Silver 1982), neonatal death may occur because of malfunction of immature organs. Examples of some of the changes that occur are: physical, such as closure of the ductus arteriosus and foramen ovale; and functional, such as production of surfactant, the development of glucose homeostatic mechanisms, and changes in the structure of haemoglobin from the fetal to the adult form.

If premature induction with the exogenous hormones bypasses some of the endocrine changes that normally occur, the newborn may be unprepared. This has been demonstrated when goat kids, which were born after the injection of $PGF_{2\alpha}$, were compared with those that were born after induction with ACTH (Currie & Thorburn 1973). The link between parturition and maturation of the fetus appears to be related to the adrenal cortex and the prepartum surge of fetal cortisol (Liggins 1978, Silver 1992, Fowden et al. 2016).

During its intrauterine life the fetus is in a thermally neutral environment, but at birth it has to be able to maintain its own body temperature. The mechanisms which enable this are the accumulation of brown fat and glycogen in late gestation and maturation of the thyroid gland. The latter process occurs as a result of the prepartum rise in fetal cortisol, which stimulates the monodeiodination of the thyroid hormones, thus enhancing their biological activity (Liggins et al. 1979, Fowden 1995).

The maintenance of glucose homoeostasis immediately after birth, when the newborn loses its placental source of glucose, is dependent on adequate stores of liver glycogen. There is strong evidence that in sheep the stimulus for the accumulation of glycogen stores in late gestation is the rise in fetal cortisol (Jost et al. 1966; review by Fowden 1995). The glycogen stores are just sufficient to provide energy before sources of glucose become available from food. A similar stimulus to the production of insulin by the pancreas has also been attributed to the effect of elevated fetal cortisol, which enables the newborn to respond quickly to maintain glucose homoeostasis.

The fetal adrenal medulla also shows evidence of maturational changes in that its ability to produce catecholamines, especially adrenaline, is increased in response to asphyxia (Comline & Silver 1971, Hooper 1995) and hypoglycaemia (Fowden et al. 1998). There is also evidence that adrenaline, together with fetal ACTH and cortisol, stimulates lung maturation, and it promotes resorption of fetal fluids within the lung, thus enabling normal respiratory function to occur upon birth.

Although it is usually possible to predict approximately when parturition will occur in domestic species, there are obvious advantages in being able to predetermine when the event will occur. Many of the methods that are used have originated from studies of the endocrine changes responsible for the initiation of normal parturition. Since the first published reports of preterm induction by Van Rensburg (1967) in the ewe and Adams (1969) in the cow, a large number of successful reports have been published in these two species as well as in the doe, sow, and mare; recently, also some success has been achieved in the bitch and the cat.

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Mare

The indications for the preterm induction of foaling are few, the main one being to ensure that it occurs in the presence of skilled assistance; then if dystocia occurs it is possible quickly to correct the difficulty so as to ensure survival of the foal and reduce the danger to the mare. There are also a few occasions when, because of disease or illness in the mare, it may be advantageous for preterm foaling to be induced. The main problems with this in mares are in fact not related the effectiveness of the agents used (see later in this chapter) but with estimating the optimum time for the birth of the fetus, given the wide range of normal gestation lengths. Even when induction takes place close to an estimated time based on the time of mating and ovulation, as well as physical changes in the mare, foals can be premature or dysmature at birth. Therefore it is recommended to examine the degree of relaxation of the sacrosciatic ligaments and the cervix and to measure the ionic composition of mammary secretions (as signs of readiness for birth) before a treatment is initiated.

A number of different hormone preparations have been used. Early reports (Britton 1972, Purvis 1972) described the successful induction of over 1500 foalings with an intramuscular injection of very high doses of oxytocin, either with or without priming with stilboestrol dipropionate. If the cervix showed evidence of 'ripening', i.e., was soft on palpation and able to allow the insertion of one or two fingers into the external os, and the foal was in the normal disposition for birth, oxytocin was given at a dose of 120 IU to mares between 360 and 600 kg live weight. Foaling occurred 15 to 60 minutes later. Purvis (1972) recorded no difficulty with placental retention, although this was a problem in the cases described by Rossdale and Jeffcoat (1975). A relationship between the dose of oxytocin and placental separation has been demonstrated (Hillman 1975); doses of < 60 IU resulting more often in retention.

Close to term, much lower doses of oxytocin (< 10 IU) appear effective (Pashen 1980, Chavatte-Palmer et al. 2002). In a more recent study (Villani & Romano 2008) 174 standard-bred mares were treated with daily intramuscular injections of 3.5 IU oxytocin once the calcium concentrations in mammary secretion had reached a level of ≥ 200 ppm; another 176 mares were allowed to foal spontaneously. Of the treated mares 68.9% foaled within 2 hours after the first (51.3%), second (14.2%) or third (3.4%) daily injection. No significant differences were found in gestational length, duration of foaling, incidence of dystocia, and incidence of retained fetal membranes between the treated and untreated mares. Induction of foaling with oxytocin is based on a direct stimulating effect on the myometrium and indirectly by stimulating uteroplacental prostaglandin release (Pashen & Allen 1979). There is evidence that intracervical application of prostaglandin E2 to ripen the cervix before induction favours a shorter delivery (Rigby et al. 1998).

In the experience of one of the authors, dystocia has sometimes occurred at induced foalings due to the lateral position of the foal (see Chapter 14), as a result of incomplete rotation before the onset of the second stage of parturition. Furthermore, foal viability can be poor due to anoxia during expulsion because of premature placental separation. It is important to know the precise gestational age because induction should not be attempted before 320 days; even then, because of the wide variation in the gestation length in the mare (as already stated previously), foal viability is poor.

Dexamethasone, a quick-release synthetic corticosteroid, has been used successfully to induce foaling in ponies (First & Alm 1977) and large 'saddle-type' mares (Alm et al. 1975). A dose rate of 100 mg every day for 4 days resulted in parturition 6 to 7 days after the start of treatment in the latter type, although the ponies responded more rapidly. The regimen was started at 321 days of gestation, with satisfactory foal survival and subsequent growth rate. The inconvenience of the repeated dosing and the delay and unpredictability in completion of foaling makes this method unattractive to clinicians.

$PGF_{2\alpha}$ and its analogue fluprostenol have also been used to induce foaling (Ousey et al.1984, Ley et al. 1989). A single dose of $PGF_{2\alpha}$ is not always effective; quite often, it is necessary to use repeated injections of 1.5 to 2.5 mg every 12 hours. In some cases these prostaglandins can cause serious discomfort and can result in a high incidence of dystocia due to abnormalities in the position of the foal (Allen 1980). Fluprostenol will successfully induce foaling between 322 and 367 days of gestation when given as a single dose of 250 µg to ponies, and 1000 µg to thoroughbred mares (Rossdale et al. 1976). The time from injection to the onset of second stage labour ranged from 33 to 183 minutes; the second stage lasted 5 to 33 minutes, and the placenta was shed by 112 minutes. The viability of the foals was generally good, although a number suffered rib damage.

Cow

The indications for the preterm induction of calving are as follows:

- Advancing the time of calving to coincide with the availability of suitable pasture for milk production. This is used in New Zealand and parts of Ireland. In the latter country it is important that the cows calve over a period of 3 months as close to 23 March as possible (O'Farrell 1979). O'Farrell has calculated that for every day that a cow calves after this date the yield is reduced by an average of 6.4 litres per day; he found that over 40% of the cows were in this category. This indication for induction might face farmers and their veterinarians with an ethical problem, in cases in which the induction results in the birth of an immature or premature calf that will not survive after birth.
- Ensuring that cows calve at a predetermined time when skilled assistance is available so that prompt attention can be given. This should reduce calf mortality and injury to the cow.
- Reducing the birth weight of the calf by shortening the length of gestation. During the last weeks of gestation the growth rate of the calf is rapid; in some of the exotic breeds, such as the Charolais, the live weight of the calf can increase by between 0.25 and 0.5 kg per day. Thus if the dam is immature with a small pelvis or pregnancy is prolonged beyond 280 days, as occurs in some more exotic breeds, the calf may be too large to traverse the birth canal. Premature induction can thus reduce the likelihood of dystocia due to fetomaternal disproportion (see Chapter 15). With this indication the stage at which calving is induced must be a compromise between the birth of the calf which is small enough to be born unaided and yet is large enough to be viable, and subsequently to have an adequate growth rate. This threshold weight will vary from breed to breed. In the pluriparous Aberdeen Angus and Hereford cow it is 40 kg, for the pluriparous Friesian 42 to 45 kg, for the 2-year-old Charolais 35 kg, and for the 3-year-old Charolais 40 to 45 kg (Meniscier & Foulley 1979).
- In diseased or injured cows in which the termination of pregnancy will alleviate the condition or in which a live calf can be obtained before slaughter, premature induction may

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be used. Cows suffering from hydrallantois will frequently respond.

A number of different hormones have been used successfully to induce calving so that a live calf is born. Because induction before 95% of the breed's normal gestation length has been passed will usually result in the birth of a small, weakly calf with poor prospects of survival (Nakao 2001), it is important that the date of service or insemination is accurately known. For the Holstein-Friesian breed, calves delivered before days 260 to 265 have a poor prognosis for spontaneous survival.

Although ACTH has been used to induce calving, because it exerts its effect by stimulating endogenous corticosteroid production, it is best replaced by the direct administration of corticosteroids. A number of potent synthetic ones are available. There are three main categories; these are referred to as long-acting, medium-acting, and short-acting, their classification being based upon the duration of the latent period (time interval from treatment to effect). Thus when given at a normal therapeutic dose rate, the long-, medium-, and short-acting corticosteroids have latent periods of 11 to 18, 5 to 11, and 1 to 6 days, respectively (Parkinson 1993). It is important to give large breeds of cows an adequate dose; in the case of betamethasone, up to 35 mg is necessary in the Charolais. Corticosteroids are also immunosuppressive, and thus they should not be given without broad spectrum antibiotics if infection is present; the lungs and udder should be carefully examined beforehand.

PGE_1, PGE_2, $PGF_{2\alpha}$, and analogues of the latter have been used. In the first reported use of prostaglandins (Zerobin et al. 1973), a minority of calvings were described as being associated with 'explosive expulsions', a 42% incidence of dystocia due to poor cervical dilation was also reported by Hendricks and colleagues (1977). However, both $PGF_{2\alpha}$ and the analogues have been successfully used from about 275 days of gestation with a latent period of 2 to 3 days (Kordts & Jöchle 1975, Day 1977, Breeveld-Dwarkasing et al. 2003a, Shenevai et al. 2012).

Good results have been obtained by using a combination of corticosteroid and prostaglandin. Beal and associates (1976) injected $PGF_{2\alpha}$ if no effect had occurred 40 hours after treatment with dexamethasone. Day (1978) obtained good results using a prostaglandin analogue, cloprostenol, administered 8 or 12 days after pretreatment with dexamethasone trimethylacetate; all the cows calved within 72 hours. In a similar trial involving 26 adult Friesian cows ranging in gestation from 237 to 270 days, 20 mg of dexamethasone phenylpropionate was given and induced calving in 13 cows on average 5.6 days later. Those that failed to respond received 500 µg of cloprostenol after 10 days, and all calved within 3 days; all live born calves survived (Murray et al. 1982). In summary, for early induction (250–270 days of gestation, when the placenta might still contribute to progesterone production), a long-acting corticosteroid is administered followed by a short-acting corticosteroid or $PGF_{2\alpha}$ after 8 days if calving has not occurred; the latent period is about 48 hours. After 270 days a medium-acting corticosteroid, with either a short-acting corticosteroid or $PGF_{2\alpha}$ after 8 days if the cow has failed to calve, is used. After 275 days, $PGF_{2\alpha}$ or short- or medium-acting corticosteroids are effective on their own. Experimental use of the progesterone receptor antagonist aglepristone demonstrated that, although calving could be initiated with complete opening of the cervix, expulsion of the calves was impaired, apparently because of insufficient myometrial contractility (Shenevai et al. 2010).

However, there are some disadvantages of preterm induction of calving:

- It is not always effective.
- The birth weight of the calf is lower than it would have been at term.
- There is also a high incidence of retained fetal membranes, up to 53% when 'short-acting' preparations are used (Wagner et al. 1971), although it is less common after the use of slow-release preparations (Welch et al. 1973, O'Farrell & Crowley 1974). Even inductions close to term pregnancy may still result in a high incidence of placental retention (Hartmann et al. 2013).
- Milk yield is initially affected, with a delay in reaching peak lactation, although there appears to be very little influence on the overall yield (Bailey et al. 1973, Welch et al. 1977, O'Farrell 1979).
- Subsequent fertility is fairly normal (Mansell et al. 2006, Nakao 2001), although the calving to conception interval and the number of services per conception are slightly increased in those cows that retain their fetal membranes.
- There is a reduction in the quality and quantity of colostral immunoglobulins, especially after the use of slow-release corticosteroid preparations, although it is unlikely that the calf will not acquire an adequate passive immunity.

Sow (See Also Chapter 19)

On average 5% to 7% of all piglets are stillborn, and it is estimated that 75% of stillbirths occur during parturition. The time interval between the birth of the first and last piglets is associated with the stillbirth rate (Randall 1972, van Dijk et al. 2005), so that prolonged farrowing results in an increase. Probably 80% of the stillbirths occur in the last third of the litter to be born.

It has been shown that the time interval between the births of two live piglets is generally shorter than the time interval which precedes the birth of a stillborn piglet (van Dijk et al. 2005). The death of the piglet may have been due to a delay in expulsion, although there is good evidence that dead piglets are expelled more slowly. One of the causes of stillbirth is the premature rupture of the umbilicus; surveys have shown that 94% of stillborn piglets had ruptured umbilical cords at birth, compared with 39% when all piglets were included (Randall 1972).

The stillbirth rate can be reduced by intensive care and attention during farrowing (Holyoake et al. 1995); however, the time of farrowing can be difficult to predict. For this reason the induction of farrowing of groups of sows at predetermined times has many attractions because this can enable a skilled person to be in attendance to a group of sows during normal working hours. Because there is also evidence that delayed or prolonged parturition can increase the stillbirth rate, then methods which accelerate the process, or at the very least prevent delays, have obvious attractions. There are also a number of other management requirements that have stimulated the application of methods to plan and regulate the timing of farrowing:

- Group farrowing facilitates multiple suckling and allows crossfostering to take place. Thus the piglets from sows with large litters or agalactia have a greater chance of surviving and being reared.
- Groups of sows and litters can be managed on an 'all in, all out' principle, thus enabling disinfection and cleaning to be performed more efficiently.
- Group farrowing facilitates group weaning.
- Farrowing can take place on certain days of the week and during normal working hours; as well as reducing the stillbirth

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rate, it can reduce the death rate due to overlaying of newborn piglets.
- It might increase the reproductive efficiency by reducing the farrowing interval by a few days.

Although synthetic corticosteroids have shown to be effective for preterm induction of farrowing, there are cheaper and more attractive alternatives for treatment (Guthrie 1985). Since the first reports of successful induction of farrowing using $PGF_{2\alpha}$ (Diehl et al. 1974, Robertson et al. 1974) or a synthetic analogue (Ash & Heap 1973, Willemse et al. 1979), a large number of reports have been published worldwide. It is now an accepted procedure in the pig industry. More recently, attempts have been made to improve its efficiency by combining prostaglandins with other hormones to even further reduce the interval between treatment and onset of expulsion of piglets.

The basic procedure using prostaglandins is that either natural $PGF_{2\alpha}$ or a synthetic analogue, such as cloprostenol, is injected intramuscularly on days 111 to 113 of gestation; farrowing will occur on average 28 hours later. Thus, if the timing of injection is between 0800 and 1000 hours, the majority of sows will farrow during normal working hours. Adjustments have to be made for herd variations in the average gestational length. In general, the earlier the time of induction, the lower the birth weights; however, piglets delivered after PG induction at 92% gestational length were behaviourally immature and few survived after 24 hours (Silver et al. 1983). If attempts are made to delay the time of induction in order to obtain larger birth weight, the degree of synchronisation is not as good.

In an interesting study involving the use of dinoprost to induce farrowing on 229 occasions (Young & Harvey 1984), it was found that 95% of farrowings occurred within 48 hours of injecting dinoprost, the majority (76%) within 24 to 36 hours, which corresponded with normal working hours on the farm. Those sows that did not farrow within 48 hours were deemed not to have responded to the injection of prostaglandin. Apart from very small litters (up to five piglets), litter size had no effect upon the response to prostaglandin; however, the duration of farrowing was directly related to litter size.

There is apparently no difference in the efficiency of $PGF_{2\alpha}$ (dinoprost) or the analogue cloprostenol, although it is generally recognised that side effects such as biting cage bars and increased respiration rate are greater after natural $PGF_{2\alpha}$ (Boland et al. 1979, Einarsson et al. 1981, Widowski et al. 1990). Recently, the use of vulvar injections of a split-dose (50% of the label dose at a 6-hour interval) was recommended to obtain a higher proportion of responding sows (De Rensis et al. 2012).

Improved expulsion of the piglets was observed when cloprostenol was combined with oestradiol benzoate. Bonte and colleagues (1981) reported that the best expulsion occurred when 10 mg of oestradiol benzoate was given 24 hours before cloprostenol, although there was some improvement when 1 mg of oestradiol benzoate was given 5 to 6 hours after cloprostenol. Kirkwood and Thacker (1995a) treated sows with 3 mg oestradiol on day 112 followed by 10 mg $PGF_{2\alpha}$ on day 113 and found that 38% of sows farrowed before 0800 hours on day 114 compared with 5% in the control group. It should be noted that within the European Union the use of oestrogens has been legally banned.

The induction with prostaglandins is based on its luteolytic effect, with the peripheral plasma progesterone rapidly declining within 12 hours after injections. Yet natural prostaglandin $F_{2\alpha}$ also causes a temporary stimulation of the myometrium and oxytocin release (Ellendorff et al. 1979b), and this even results in a transient hypoxia of the piglets in utero (Randall 1990). Oxytocin has also been used routinely in combination with $PGF_{2\alpha}$ as part of induction protocols, the most common approach being to administer it 24 hours after prostaglandin. This usually resulted in a significant reduction in the interval to farrowing, although the response may be related to the litter size (Chantaraprateep et al. 1986). Others have used repeated injections of oxytocin (Zarro et al. 1990). It is important that dose rates of oxytocin of about 10 IU should be used because higher dose rates will cause myometrial spasm and may even delay farrowing. In a study in which 20 IU of oxytocin was given at 24 hours after PG administration, a high proportion of manual intervention was needed for those sows that had the best synchrony of the onset of delivery (Dial et al. 1987).

Ewe

Parturition can be induced in the ewe by means of corticosteroids or by drugs that will either inhibit the synthesis of progesterone (such as epostane) or block progesterone at the receptor level. The indications for preterm induction are limited because dystocia due to fetomaternal disproportion is not as common as in cows (see Chapter 15). However, a system that can guarantee that lambing will occur only during the hours of daylight when skilled assistance is available might reduce any problems due to dystocia and increase lamb survival rates. However, it is not possible to shorten gestation length by more than 7 to 10 days without increasing lamb mortality.

As with other species, an accurately known gestational age is important. When corticosteroids such as dexamethasone, flumethasone, and betamethasone are given by a single intramuscular injection within 5 days of term, normal parturition occurs in 2 to 3 days (Bosc 1972, Bosc et al. 1977, Penning & Gibb 1977, Kastelic et al. 1996). Cahill and associates (1976), using similar methods, had a higher than normal incidence of dystocia with poorer lamb survival. Unfortunately, attempts to concentrate lambing during the hours of daylight by premature induction with corticosteroids have not been very successful (Bosc 1972). Induction of lambing in groups of ewes that have been synchronised with progestogens (see Chapter 8) can ensure that ewes lamb over a relatively short period of time in a single batch. When the 3β-hydroxysteroid dehydrogenase inhibitor epostane was given (orally, intravenously, or intramuscularly) between days 136 and 142, birth of viable lambs occurred between 33 to 36 hours after application (Silver 1988). The intramuscular injection of the progesterone receptor antagonist RU 486, both on day 144 and 145 days of pregnancy, significantly advanced the birth of viable lambs (Gazal et al. 1993). Recently, Özalp and colleagues (2017) demonstrated that two injections (on day 140 and 141) of aglepristone (either 5 or 10 mg/kg body weight) could precisely control the lambing time without any adverse side effects in either mothers or lambs.

Doe (Nanny) Goat

Preterm induction of parturition in goats can be achieved by using a luteolytic dose of natural prostaglandin $F_{2\alpha}$ (Romano et al. 2001) or its synthetic analogues (Batista et al. 2008). Some 80% of the treated animals delivered their kids between 30 and 40 hours after injection given on day 145 of gestation. Dystocia and retention of the fetal membranes did not occur.

Bitch and Queen Cat

Because of side effects and the need for repeated treatment, $PGF_{2\alpha}$, is not an attractive option for the induction of parturition at term. The steroid enzyme inhibitor epostane, the prolactin inhibitors

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cabergoline and bromocriptine, and progesterone receptor blocking agents have been used successfully for the induction of abortion in dogs and cats (Lamm & Makloski 2012). Studies by Fieni and colleagues (2001) and Baan and colleagues (2005) demonstrated that progesterone receptor blocking agents such as mifepristone and aglepristone can also be used for the induction of whelping, without any adverse effects on the progress of delivery, birth weights, and pup survival rates. However, in a more recent study with different breeds (Fontbonne et al. 2009) the duration of the mean interval between two successive expulsions was significantly longer in the induced group than in the control group.

Accelerating Parturition

Oxytocin, administered as a bolus injection, has long been used to treat sows that appeared to be suffering from uterine inertia as shown by prolonged farrowing times. In such cases this treatment should always be preceded by a vaginal exploration to detect if a piglet within the pelvic canal or a piglet in faulty disposition cranial to the pelvic inlet is the primary cause of the prolonged farrowing. Routine use of oxytocin 1 to 2 IU used repeatedly in delayed farrowing was effective in accelerating the process. Doses in excess of 10 IU induce uterine spasm and are contraindicated (Zerobin 1981, Taverne 1982). Depot or slow-release oxytocin preparations are not effective.

Studies by Pejsak and Tereszczuk (1981) in which 10 IU of oxytocin was given to 836 primiparous and pluriparous gilts and sows by the intramuscular or intranasal route as a routine procedure (during apparently normal parturitions and in the absence of prolonged farrowing) demonstrated some favourable results. In their regimen, oxytocin was given immediately after the birth of the first piglet and was repeated if there was a delay of more than 1 hour before the birth of the next piglet. If no response was observed in 10 to 20 minutes, a vaginal examination was performed. All the oxytocin-treated sows farrowed within 10 hours, although some in the control group exceeded this duration. However, more recent studies (Mota-Rojas et al. 2002, 2005, Alonso-Spilsbury et al. 2004), using injections of 20 to 50 IU of oxytocin (depending on maternal body weight) after the birth of the first piglet, clearly demonstrated that such treatment increases stillbirth rate, despite a significant shortening of the expulsive phase.

Apart from oxytocin, a wide range of other drugs have been tested in the past to control the timing and speed of delivery in piglets (review by Guthrie 1985) but very few of these treatments have become standard procedures. A beta-blocking agent, carazolol, has been used to shorten the duration of farrowing with encouraging results. The rationale behind its use is that during pregnancy, because ß-receptors in the uterus become dominant, the stress resulting in adrenaline release will cause relaxation of the uterus. If these receptors are blocked with carazolol, then in stressed animals (especially gilts) the adrenaline will have little or no effect upon the myometrium, the uterus will retain its tone, and parturition will not be delayed (Taverne 1982). In a double-blind trial involving 1000 sows using a dose rate of 0.5 mg/50 kg given at the beginning of labour, there was a significant reduction in the duration of farrowing ($P < 0.05$), a reduction in the stillbirth rate, and in the incidence of obstetrical complications, especially in gilts (Bostedt & Rudolf 1983). However, a study in which parturition was induced on day 113 with a PGF analogue and was followed (24 hours later) by an injection of the beta-blocking agent propranolol failed to show any effect on the onset or duration of parturition (Kirkwood & Thacker 1995b).

Delaying Parturition

ß-Adrenergic Agents

As already mentioned previously, delaying parturition has been accomplished in sheep, goats, and pigs (but not in mares) by the pharmacological inhibition of prostaglandin synthesis. In pigs this delay was based on the postponement of prepartum luteolysis (Taverne et al. 1982).

ß-Adrenergic agents, which stimulate the $ß_2$ receptors of uterine muscle cells, can abolish uterine contractions and delay parturition for a short time. One such substance, clenbuterol, has been used successfully in cows, sows, and ewes (Ballarini et al. 1980, Collins et al.,1980, Jotsch et al. 1981, Garcia-Villar & Toutain 1991). In cows, provided that the cervix is not fully dilated and second stage has not commenced, an injection of 0.3 mg of clenbuterol hydrochloride (10 ml), followed by a second injection of 0.21 mg (7 ml) 4 hours later, will inhibit calving for 8 hours after the second injection. As well as being used as a management tool, it can be effective in ensuring improved relaxation of the vulva and perineum in heifers. When the drug was given to cows in labour, it inhibited myometrial activity only briefly (< 3 hours), without adversely affecting fetal outcome (Jonker et al. 1991). In Welsh mountain pony mares, intravenous injections of clenbuterol failed to delay parturition (Palmer et al. 2002).

In sows clenbuterol causes relaxation of the myometrium and hence interrupts expulsion of the piglets. After several hours there is a return of spontaneous myometrial contractions without any adverse effects upon the viability of piglets. Zerobin (1981) obtained good responses with a dose rate of 150 µg. He also reported that the use of oxytocin will reverse the effect of the clenbuterol. In pregnant and parturient bitches, intravenous injections of the calcium-channel antagonist nifedipine were effective in inhibiting the mechanical and electrical activity of the myometrium in vivo (van der Weyden et al. 1989).

Course of Parturition: Physiology

The essential components of the birth process are the expulsive forces, the fetus(es) together with their associated fetal membranes and fluids, as well as the soft and bony parts of the birth canal. Normal birth will result when these forces are sufficient to expel a normal, correctly disposed fetus in the correct disposition (see Chapter 14) and fetal membranes through a dilated cervix into the birth canal of adequate dimensions.

The expulsive forces consist of the contractions of the myometrium and the abdominal musculature; the relative importance of these two components varies from species to species. During pregnancy the fetus occupies as small a space as possible; in order to do this it flexes its limbs and neck and, particularly in the monotocous species, assumes a disposition so that its dorsum is adjacent to the greatest curvature of the uterus. In order to negotiate the birth canal, the fetus must be correctly disposed so that its body conformation is as 'streamlined' as possible to match the shape and direction of the birth canal; this is particularly important in monotocous species. Finally, the birth canal must allow the passage of the correctly disposed fetus. Changes occur in the maternal structures that allow this to occur. The cervix must dilate, the bony pelvis and its related ligaments must relax, and the vagina, vulva, and perineum must soften. For extensive reviews on the comparative physiology of parturition, with special reference to the control of myometrial and cervical functioning, the reader is referred to: Gibb et al. (2006), Zakar & Hertelendy

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2007, Taverne & van der Weijden (2008), Aguilar & Mitchell (2010), and Yellon (2017).

Myometrial Contractions

The hormones that bring about some of these changes have already been discussed; however, the mechanisms involved in the myometrial contractions have not. The myometrium is formed from two main types of muscle protein, namely myosin and actin. The contractions of the myofibrils occur because of the formation of covalent crosslinked bonds between the actin and myosin filaments. Contractions are initiated when the ATPase of myosin light chain (MLC), which is one of the myosin components, is phosphorylated by the action of MLC kinase (MLCK). This enzyme, MLCK, is activated by the calcium-binding protein Calmodulin. Myometrial relaxation occurs because of dephosphorylation of MLC by the action of MLC phosphatase or cAMP-dependent protein kinase that inhibits MLCK, and hence phosphorylation of MLC. It is important to consider the structural arrangement of the smooth muscle bundles. In domestic species with a bicornuate uterus, the myometrium comprises two layers. In the outer layer the bundles are arranged parallel to the long axis of the uterus, so that when these contract, the uterus is shortened cephalocaudally. The myometrium is continuous with the cervix, which is fairly well secured within the pelvic cavity; thus when the longitudinal bundles contract, the uterine horns will be pulled caudally. When the uterus contains a full-term fetus, the ability of the uterus to shorten is reduced and, as a result, the contractions may cause some dilatation of the cervix. The inner layer of the myometrium is formed by bundles of fibres arranged concentrically around the longitudinal axis; thus these contractions will constrict the uterine lumen (Porter 1975).

During pregnancy, as a result of the stimulus of both progesterone and oestrogens on protein synthesis and the localised influence of distension by the developing conceptus, there is hypertrophy of the myometrium. In early pregnancy there is also some evidence of hyperplasia. As a result of this, the length of the myofibrils is increased ten-fold, and the width two-fold. Because the myometrial mass is increased, its work capacity is increased. Progesterone is necessary for the stretch-induced hypertrophy, but during the final days of gestation, when progesterone levels decline and/or progesterone effects subside, there will be discordance between fetal growth and uterine size, resulting in increased wall tension and formation of gap junctions between smooth muscle cells (Gibb et al. 2006). As well as the physical changes in the myometrium, there are also changes in the electrophysiology of the smooth muscle. It has been demonstrated that in many species during pregnancy, there is an increase in the resting membrane potential. With the prepartum rise in oestrogens and the removal of the progesterone block, there is discharge of action potentials and the initiation of myometrial contractions. The resting membrane potential of the oestrogen-dominated myometrial cell is also close to the threshold level for the spontaneous firing of action potentials. Thus if the myometrium is stretched, there is slight depolarisation and discharge of action potentials. In rats mechanical stretching induces the expression of several contraction-associated proteins (CAP's) such as Connexin-43 (involved in gap junction formation) and oxytocin receptors, and these effects could be antagonised by progesterone. These findings may have clinical implications for the progression of the expulsive stage, especially in polytocous species when embryonic or fetal mortality has resulted in more or less empty, i.e., nondistended segments within a uterine horn.

Effects of Progesterone and Oestrogens on Myometrial Activity

Progesterone dominance during pregnancy ensures that the myometrium remains relatively quiescent, although there is some evidence in some species that relaxin and PGI_2 may also play a role (see later in this chapter). Oestrogens have the reverse effect. Although the mechanisms involved are not fully understood, it has been suggested that oestrogens might exert their effect by: (1) increasing contractile protein synthesis; (2) increasing the number of agonist receptors for oxytocin and prostaglandins; (3) increasing calmodulin synthesis; (4) increasing MLCK activity; and (5) increasing the number of gap junctions, which are low-resistance pathways for the transmission of electrical and molecular information between smooth muscle cells. Oestrogens, in this way, increase the efficiency of the myometrium as a contractile unit. Progesterone has the opposite effects. It: (1) reduces the number of gap junctions; (2) reduces the number of agonist receptors; (3) inhibits the synthesis of prostaglandins and the release of oxytocin; and (4) increases calcium binding.

Role of Prostaglandins and Oxytocin

Prostaglandins play a critical role in parturition, not only in the initiation of the process but in the control of myometrial contractions. These actions are facilitated because the molecular structure of prostaglandins enables them to move freely through extracellular fluids and lipid cell membranes. In general, $PFG_{2\alpha}$ and PGE_2 are responsible for stimulating uterine contractions (although this may depend, at least in some species, on the localisation within the pregnant uterus), whereas PGI_2 inhibits uterine contractions (Omini et al. 1979). Availability of these prostaglandins within the uterine tissues depends on the relative expressions of enzymes involved in their synthesis and metabolism, and these are under the control of wide range of different steroids, peptide hormones, and cytokines. In general, the expression of enzymes catalysing the synthesis of PGF in uterine tissues is upregulated during parturition, whereas that of enzymes involved in PG inactivation are downregulated (Vannuccini et al. 2016; Fig. 6.7). Prostaglandins actions are mediated through specific receptors, and, as a consequence, they influence the number of gap junctions and also the intracellular mobilisation of Ca^{2+} of smooth cells; $PGF_{2\alpha}$ and PGE2 enhance and PGI_2 inhibits these changes.

The pattern of oxytocin release during late pregnancy and parturition has been studied in the ewe (Fitzpatrick 1961, Glatz et al. 1981), goat (Chard et al. 1970, Hydbring et al. 1999), mare (Allen et al. 1973, Vivrette et al. 2000), cow (Schams & Prokopp 1979, Hydbring et al. 1999), sow (Forsling et al. 1979a & b, Gilbert et al. 1994), and dog (Olsson et al. 2003, Klarenbeek et al. 2007). It is interesting that in all these species the oxytocin levels during late pregnancy and the early stages of parturition remain fairly low. In the cow, sheep, and goat, they rapidly increase to peak values at the time when the fetal head emerges from the vulva and when the fetal membranes are expelled. In the mare the first significant increase of oxytocin in samples taken from the intercavernal sinus was detected between 18 and 72 hours before delivery of the foal, clearly after an increase had already taken place in the plasma dihydro-15-keto $PGF_{2\alpha}$ levels (Vivrette et al. 2000). In pigs they only increase shortly before the start of the expulsive stage, remain elevated, but with considerable pulsatile fluctuations, during delivery of the piglets and decline thereafter. In dogs the release pattern still needs further investigation because pulses were detected in conjunction with, but also in the absence of, the birth of a pup (Klarenbeek et al. 2007). So at least in some

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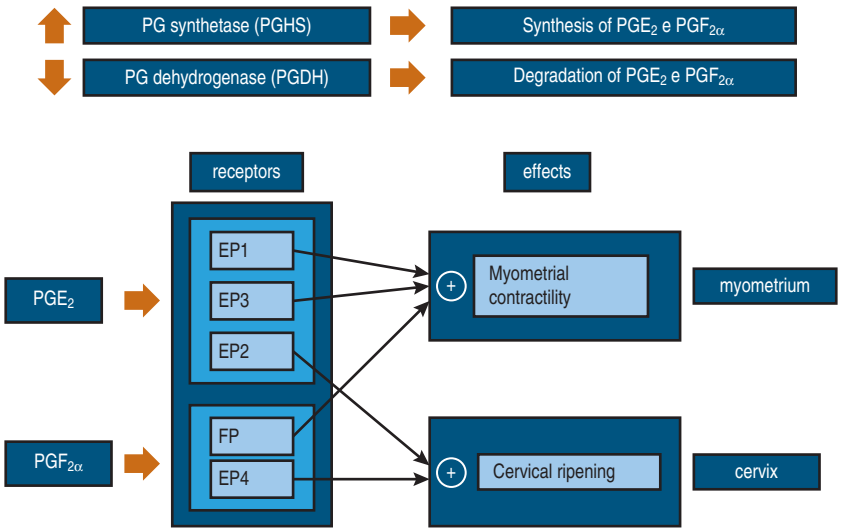
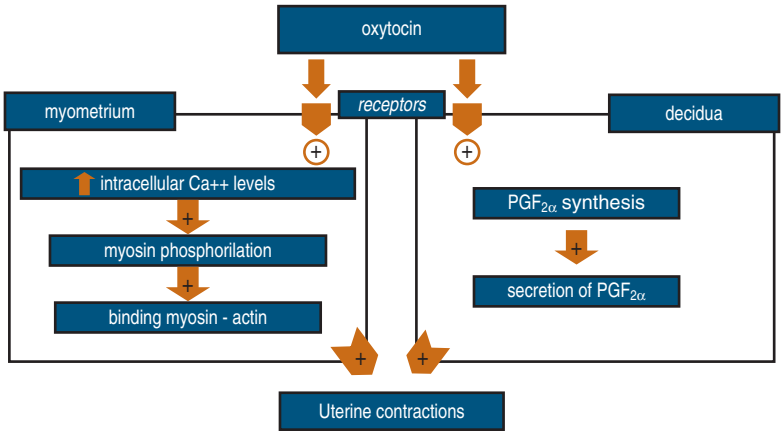


• **Fig. 6.7** Prostaglandin effects on myometrium and cervix at parturition. (From Vannuccini et al. 2016.)



• **Fig. 6.8** Oxytocin effects on myometrial contractility. (From Vannuccini et al. 2016.)

of the species, oxytocin plays only a minor role in the initiation of uterine contractions.

The main release of this hormone occurs as a result of the stimulation of sensory receptors in the anterior vagina and cervix (Ferguson's reflex). There is certainly a good correlation between electromyographic activity and oxytocin release in the sow (Ellendorff et al. 1979a), which is suggestive of a local, positive feedback mechanism operating in the uterus of this species.

Oxytocin receptors increase during late gestation and with the onset of parturition; this is dependent mainly on the decline in progesterone and increase in oestrogens (pig: Lundin-Schiller et al. 1996; sheep: Wathes et al. 1996; cow: Fuchs et al. 1992). Little is known in domestic species about their distribution in the circular and longitudinal muscle layers. Oxytocin stimulates uterine contractions in two ways (Vannuccini et al. 2016; Fig. 6.8): first, by increasing prostaglandin release, with which there is a synergistic effect; second, by increasing Ca^{2+} release from the endoplasmic reticulum that increases MLCK phosphorylation (MacKenzie et al. 1990). An integrated view on oxytocin and its participation in the regulation of pregnancy and parturition is given by Burbach and colleagues (2006).

Stages of Labour

From a physiological point of view, parturition has been subdivided into four phases (Gibb et al. 2006):

- Phase 0 comprises some 95% of the duration of gestation, with the myometrium more or less quiescent and with the cervix hard and unyielding.
- Phase 1 is the activation phase, during which rapid softening of the cervix takes place and the myometrium becomes prepared for contractions to occur. This is induced by increased expression of CAP's, which occurs as a result of the removal of inhibitory factors (such as progesterone, relaxin), an increase of uterotrophic factors (such as prostaglandins, oestrogens), and increased stretching of the uterine wall. Thus this phase comprises all of the final endocrine events by which the fetal initiating signals are translated into functional changes in the cervix and myometrium.
- Phase 2 the stimulation phase, involves the first and second stages of labour (see later in this chapter) and is characterised by increasing myometrial contractility (by steep increases of PG in oxytocin release), dilatation of the cervix, and expulsion of the fetus(es).

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- Phase 3 comprises the afterbirth stage (but in fact only in those species in which the fetal membranes are expelled one or more hours after the fetus) and the start of uterine and cervical involution (see Chapter 7).

More traditionally, and from a more practical perspective, the process of parturition has been divided into three separate stages which are referred to as 'the stages of labour'. Although it is convenient to consider the process in this way, it is important to remember that these stages do not start and end abruptly, but pass gradually from one to the other.

First Stage of Labour

The changes that occur during this phase of parturition are not visible externally but are important because they prepare the birth canal and the fetus for expulsion. A number of important changes occur. First, the structure of the cervix changes so that it can dilate; second, there is the onset of myometrial contractions; and finally, the fetus assumes the disposition for expulsion, which involves (in monotocous species) rotation about its longitudinal axis and extension of the extremities.

The changes in the structure of the cervix have been studied and reviewed (Fitzpatrick 1977a and b, Fitzpatrick & Dobson 1979, Dobson 1988, Breeveld-Dwarkasing et al. 2003b). They involve a loosening of the ground substance of its structure due to changes in the composition of collagen components. There is also increased incorporation of water, which permits the collagen fibres to separate from each other particularly under the extension forces, and possibly also allows ready access of previously inactive proteases to susceptible sites for the breakdown of the collagen molecules. These biochemical changes are mediated by complex interactions between cytokines and prostaglandins, as well as peptides and steroid hormones; these show many of the characteristics of an inflammatory process (see reviews by Kelly 2002, Keelan et al. 2003, Yellon 2017). In all of the species investigated so far, these processes already start under the dominance of progesterone during the second half of pregnancy.

The cervix of the cow dilates, with the external os opening before the internal os (Abusineina 1963); the same has also been reported in the ewe (Fitzpatrick 1977b). The time taken for cervical dilatation varies. In the sow, using sequential vaginal exploration, it was found that it takes between 1 hour and 2 days, with 50% taking 6 to 12 hours (Schmid 1937). In the cow the dilatation of the external os has been measured in vivo during PG-induced calvings (Breeveld-Dwarkasing et al. 2002, 2003a). It started, on average, 28.5 hours after PG injection at a rate of 2.6 cm per hour. The mean interval between the onset of dilatation and expulsion of the calf was about 9 hours. The mechanism responsible for dilatation is still not fully understood. For many years it was assumed to be a passive process brought about by the passage of the fetus and the fluid-filled fetal membranes through the ripened cervix. It has also been suggested that it is mainly an active process caused by the contraction of the longitudinal muscle bundles. More recently, it has been demonstrated that the outer smooth muscle layer of the cervix displays clear electromyographic activity during dilatation at (induced) calving, usually in conjunction with that of myometrial EMG activity (van Engelen et al. 2007). However, it has been found that in some ewes only weak myometrial contractions precede dilatation (Hindson et al. 1968), and in some women the cervix can dilate in the absence of uterine contractions (Liggins 1978). Ledger and associates (1985) demonstrated that in ewes, even when the cervix and uterus were isolated surgically, cervical softening still occurred. Thus the biochemical changes, previously described, are possibly more important than the contributions from the smooth muscle of the cervix or myometrium. These changes are probably not just the degradation of collagen, but rather a remodelling of the cervical matrix with new collagen and proteoglycan synthesis (Challis & Lye 1994). It is likely that in normal parturitions it is a combination of active and passive mechanisms.

In the cow there is initially wide dilatation of the external os, whose perimeter is palpable as a frill at the cranial end of the vagina. The cone-shaped cervix then undergoes a simultaneous shortening, before the internal os dilates, and when this has occurred, the vagina and uterus form a continuous canal which becomes tightly engaged by the distended allantochorion. In women this process has been visualised by ultrasonographic imaging, but this still awaits exploration in domestic animals.

The first stage of labour is also characterised by the onset of regular myometrial contractions, which frequently produce signs of discomfort and mild colic, the degree of response varying from species to species and from individual to individual. However, in most cases there is restlessness with elevated pulse and respiratory rates; the body temperature usually (but not consistently) falls a degree or so. In the sheep and goat during late gestation, uterine contractions, either measured by intrauterine pressure changes or by EMG activity, occur once every 30 to 60 minutes; they are of low amplitude but of 5 to 10 minutes' duration. To discriminate them from the typical labour-like contractions, they have also been labelled as 'contractures'. This pattern continues until some 48 to 24 hours before parturition; thereafter their frequency and amplitude increase and their duration decrease. It is only in the last 12 hours, and in some ewes in the last 2 hours, that clearly coordinated contractions occur at a regular frequency (30 per hour), of short duration (1 minute) and substantial amplitude (20–25 mm Hg) (Fitzpatrick & Dobson 1979). Ward (1968) found that there is a significant increase in myometrial activity during the last 4 hours before the expulsion of the lamb.

In the cow, contractures last on average some 12 minutes during late gestation, and they predominantly occur when the animals are recumbent (Taverne et al. 2002). They are gradually transformed to a more regular coordinated peristaltic type, which started (in induced calvings) some 16 hours after PG injection (Breeveld-Dwarkasing et al. 2003a). The frequency of intrauterine pressure waves increases from 12 to 24 per hour in the last 2 hours, and 48 per hour just before expulsion (Gillette & Holm 1963, Burton et al. 1987). One feature identified in the cow (Gillette & Holm 1963, Taverne et al. 1979c), which will also be discussed later in this chapter in the sow, was the presence of cervicotubular and tubular–cervical contractions. The function of the former type is not known in the cow.

The myometrial contractions in the sow are more complex, which is not surprising because each pregnant uterine horn may reach a length of 1.5 to 2 metres at the end of gestation. In sows, contractures have been found during pregnancy using EMG recordings, and they switch to a more regular and coordinated pattern between 6 and 9 hours before the birth of the first piglet (Taverne et al. 1979a). Using pressure measurements, this transition was found to occur somewhat earlier: IUP waves increased in frequency and amplitude and became more regular 12 to 72 hours before the onset of the second stage (Zerobin & Spörri 1972, Ngiam 1977). Contraction frequencies of 8 to 24 per hour and durations of 0.5 to 3.5 minutes at amplitudes up to 60 mm Hg have been recorded from about 24 hours prepartum. A change in the pattern of the contractions was observed by Zerobin and Spörri (1972) at the time that milk appeared in the teats. The same authors, using pressure

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recordings, and Taverne and colleagues (1979b), using myoelectrical techniques, identified the presence of cervicotubular and tubular–cervical contractions. The latter authors observed that once one of the horns was empty, cervicotubular contractions decreased or disappeared, yet tubular–cervical contractions remained present in the opposite horn as long as it contained piglets. Because it has been known for some time that uterine volume is important in regulating myometrial activity by altering myoelectrical activity (Csapo et al. 1963), it has been postulated that the cervicotubular contractions prevent the premature displacement of piglets, thus ensuring orderly expulsion from the horns (Taverne et al. 1979b).

In late pregnant bitches, contractures of the myometrium have been recorded using electromyography (van der Weijden et al. 1989). During the last 7 days before delivery, there is a progressive increase of more short lasting EMG bursts that is correlated with a gradual decline of the plasma progesterone levels. A significant increase of total EMG activity and burst frequency occurred between 24 and 13 hours before delivery, coinciding with a sudden fall in body temperature and plasma progesterone.

The contractions of the uterine musculature cause other changes in the uterus and probably also in the fetus. In the placenta the attachments to the endometrium become less intimate, and the superficial cells undergo fatty degeneration, whereas in those species with a deciduate placenta, separation of the margins, with haemorrhage, is beginning. Yet placental attachment normally stays intact during parturition and is only disrupted at expulsion (dog and cat) or after expulsion of the fetuses (pig, mare, ruminants). The increased resistance to blood circulation in the maternal side of the placenta during contractions causes a correspondingly reduction of blood gaseous exchange in the placenta, and this may be reflected by temporary changes in fetal P_{O_2} and fetal heart rate (Jonker et al.1996). On the maternal side this impediment to circulation may aid diversion of blood to the mammary glands.

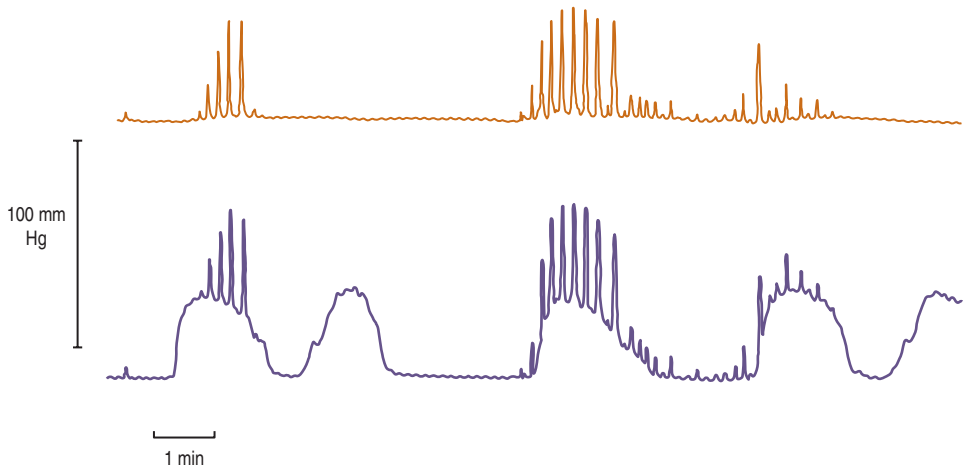
As regards the fetus in first stage labour, it becomes more active and disposes itself in a manner that will allow it to negotiate the birth canal. Thus in the foal there is a progressive rotation from the ventral to the dorsal position, whereas the forelimbs, head, and neck become extended (Jeffcott & Rossdale 1979). Also, in the case of the calf and lamb, rotation to a dorsal position and extension of the forelegs is necessary to change the fetus from its gestational position and posture to that for parturition (Fraser & Terhurne 1977). The nature of the mechanism whereby the forelimbs become straightened in front of the body is unknown. In the bovine species this is a unique attitude that is never repeated after birth. In his studies of the first stage of labour, Abusineina (1963) noticed that the flexed knees of the calf first occupied the dilating cervix and 30 minutes later the digits were in the cervix. It has been suggested that at this time the fetus is 'practising' righting reflexes and that it extends the carpal joints in its efforts to 'stand up in utero'. It is likely also that these spontaneous fetal movements occur in response to increased uterine pressure caused by the myometrial contractions during the first stage. If this view is correct, then the mother, through the medium of an indifferent myometrial function, could be partly responsible for fetal dystocia due to postural errors (see Chapter 14). With further reference to the importance of myometrial function, it is significant that, when birth is premature, cervical dilatation is often incomplete, and fetal postural defects are then common; also retention of the afterbirth is likely. All these clinical effects may be partly due to relative uterine inertia, which may result from a disordered sequence of preparturient endocrine events.

Second Stage of Labour

In the monotocous species this refers to the expulsion of the fetus; however, in polytocous species the fetal membranes are sometimes voided together with fetuses, and hence there is no clear differentiation between the two stages.

The sign of the onset of second stage is the appearance of abdominal contractions. In the cow it has been shown that 8 to 10 of these are superimposed upon the onset of each myometrial contraction, whose frequency at this stage is 24 to 48 per hour, so that one contraction is almost immediately followed by another (Gillette & Holm 1963, Zerobin & Spörri 1972). Similar observations were made in the ewe in which the frequency of contractions increased to 40 per hour, with only very short periods of rest, and intrauterine pressure was increased to 30 to 40 mm Hg with each contraction (Fitzpatrick & Dobson 1979).

In many species the superimposition of abdominal contractions upon myometrial contractions has been demonstrated; this is shown in an intrauterine pressure recording obtained from a sow (Fig. 6.9). It should be remembered that these abdominal contractions, which cause straining, are not related directly to the release of oxytocin and should not be confused with Ferguson's



• **Fig. 6.9** Pressure changes at parturition in the sow. Upper trace is from a balloon-tipped catheter placed in the peritoneal cavity. Lower trace is from an intrauterine balloon-tipped catheter.

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reflex. The coordination between the two is due to the fact that the myometrial contractions force the fetus into the pelvic inlet, which activates the pelvic reflex and stimulates straining; this is a similar response to the one that stimulates defecation. The straining forces the fetus against the cervix and anterior vagina, thus initiating Ferguson's reflex, so that the oxytocin that is released causes further contractions of the myometrium. As already indicated previously, especially in the monotocous species, it is during this stage that peak levels of plasma oxytocin are present.

The allantochorionic sac ruptures as a consequence of its caudal movement being restricted by its placental attachments, and a gush of urine-like fluid escapes from the vulva. The distended amnion, together with parts of the fetus, are forced into the pelvic inlet, thus stimulating the pelvic reflex, which induces powerful contractions of the abdominal muscle. Similar, substantial straining occurs later when first the fetal shoulders and later the fetal hips engage the pelvis. These distensions of the maternal birth canal cause great increases in the release of oxytocin from the posterior pituitary, which in turn stimulates myometrial contractions; thus there is a conjunction of uterine and abdominal expulsive efforts. As the intermittent straining continues, the amnion traverses the vagina and appears at the vulva as the 'water bag'. With further straining, fetal limbs appear in the water bag; in the case of the monotocous species, one limb slightly precedes the other. The amnion is progressively expelled and may or may not become ruptured by a fetal foot, with escape of some of the lubricant amniotic fluid. The fetal head next occupies the vulva, and at this time the contractions of the uterine and abdominal muscles reach a climax of expulsive effort, maximum effort coinciding with the birth of the fetal occiput. When the head is born, the mother may rest for a bit, but soon a further bout of straining causes the fetal thorax to pass through the vulva. Usually, birth of the hips quickly follows, and the hindlimbs may be expelled at the same time; in many cases in the monotocous species, there is no further expulsive effort after the birth of the hips with the hindlimbs remaining in the vagina until they are freed either by movement of the newborn or by the mother rising from recumbency.

If the fetus is born in the amnion, or if the partially ruptured amnion covers the face of the offspring, fetal movement quickly causes it to be ruptured or drawn away; respirations, often accompanied by a cry, then begin. There seems to be very little risk of a healthy fetus being suffocated by an enveloping amnion. The stimulus to breathing is apparently the impact of air at the nostrils and/or fetal hypercapnia, for occasionally. during the expulsive phase of equine and bovine births when the protruding face has become uncovered, breathing had commenced before delivery was completed. On the other hand, sometimes expulsion apparently occurs so quickly (especially in pigs) that the newborn lies completely immobile behind the sow and will start to breathe many seconds later.

When the mother gives birth in lateral recumbency, the offspring is often born with an intact umbilical cord, and some minutes may elapse before the cord is ruptured by movement of the young animal or mother. This is especially true at foaling. It is important to allow this to happen naturally, for artificial and premature rupture, or ligation, of the cord may deprive the newborn of a large volume of blood, which would normally pass to it from the placenta. In piglets the umbilical cord may become stretched or may even rupture during delivery (see later in this chapter). In ruminants rupture occurs close to the umbilicus, at which point the umbilical arteries and the vein have a 'weak spot' where there is an abundance of circular smooth muscle in their walls. After rupture the arteries and urachus retract towards, or into, the fetal abdomen, which prevents haemorrhage. In foals the cord also ruptures at a preformed 'weak spot' close to the umbilicus where a smooth muscle sphincter surrounds the blood vessels and urachus (Naaktgeboren & Slijper 1970).

The second stage of labour is complete when all fetuses have been delivered; it lasts from an average of 17 minutes in the mare to an average of 4 hours in the sow (see later in this chapter). Its duration and the degree of effort associated with it are usually greater in primigravida than in multigravida.

In the mare, cow, doe goat, and ewe (when monotocous), the fetus is usually delivered in anterior presentation, dorsal position, and extended posture (see Chapters 11 and 14), although a small proportion of normal deliveries may occur in posterior presentation, dorsal position, and extended posture. In the polytocous bitch, cat, and sow, up to 40% to 45% of fetuses may be delivered in posterior presentation, and thus posterior presentation represents a normal physiological phenomenon in these species. During passage of the fetus from the uterus to the exterior, the fetus follows an arched route. During the straining movements of the dam the height of the pelvic cavity is enlarged, especially by the dorsal excursion of the sacrum. This facilitates final fetal expulsion.

Third Stage of Labour

After birth of the young, regular abdominal contractions largely cease, but myometrial contractions persist. In general, they decrease somewhat in amplitude but become more frequent and regular. These contractions are important for dehiscence and expulsion of the fetal membranes. Not only do the waves of contractions passing from uterine tube to cervix persist (Taverne et al. 1979c), but in the cow there is the reappearance of contractions in the reverse direction within 10 minutes of the expulsion of the calf (Zerobin & Spörri 1972). Taverne and colleagues (1979b) reported in the sow the presence of regular contractions of a frequency of 15 to 27 per hour, which predominantly progressed in a peristaltic tubocervical fashion over the entire length of the uterus.

During the last days of gestation, maturational changes occur in the placenta; these are likely to be related to the changes in the endocrine environment that trigger parturition. Collagenisation of the placentome, flattening and loss of the maternal crypt epithelium, increased infiltration and activity of leucocytes, and a decline in the number of trophoblast giant cells in the cow have been identified (Grunert 1984, Gunnik 1984, Woicke et al. 1986, Williams et al. 1987, Peter 2013). Using mRNA microarray analysis, differential gene expression in placentomes obtained from cows before (around day 270) and during calving revealed some interesting data on placental detachment (Streyl et al. 2012). Whereas mRNA levels from genes related to mitotic and tissue differentiation were higher found in the samples from the antepartum animals, a shift to a higher expression of genes involved in apoptosis, degradation of the extracellular matrix, and the innate immune response occurred in tissue from intrapartum animals. That (failure of) immune-mediated rejection appears to be involved in placental detachment (retention) was already indicated by several earlier studies (see, for example, Gunnik 1984, Joosten & Hensen 1992, Benedictus et al. 2012). Two-way compatibility of major histocompatibility class I molecules (MHC I) of both the dam and her calf gave a higher risk of retained placenta by influencing the activity of fetal macrophages and the release of inflammatory cytokines from both maternal and fetal macrophages in the

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uterus (Benedictus et al. 2015). As a result, the maternal immune system does not reject the fetal membranes, and the fetal immune system does not raise an immune response against maternal MHC class I antigens that could influence rejection of the membranes by the mother.

Expulsion of the fetal membranes is caused by uterine contractions. The effect of the contractions is to open up the endometrial crypts, which in the case of those species with cotyledons, resemble the opening of the blades of a fan. The fetal villi shrink, due mainly to the sudden loss of turgidity related to the escape of blood from the fetal side of the placenta when the umbilical cord ruptures. Exsanguination of the placenta is also aided by the squeezing effect of myometrial contractions. These actions, together with the degenerative or maturational changes described previously cause separation and shedding of the fetal membranes. As a result, the apex of the allantochorionic sac becomes inverted, and as the sac is 'rolled' down the cornua, the fetal villi are drawn out of the crypts. When a large portion of the afterbirth becomes detached and inverted it forms a mass within the maternal pelvis that stimulates reflex contractions of the abdominal muscles; this straining completes the expulsion of the allantochorionic sac, which is seen to have its smooth, shining allantoic surface outermost. In the dog and cat the dehiscence and expulsion of the placentas are interspersed with the fetal births; but only the expulsion of the last afterbirth simulates the third stage of the monotocous species. In the sow most of the (fetal) placentas remain attached to the endometrium during expulsion of the piglets; only occasionally are fetal membranes expelled in association with, or in between, the expulsion of piglets. The bulk of the fetal membranes is normally expelled within 4 hours after the last piglet. In the mare the third stage lasts from an average of 1 hour (Steven 1982). In the majority of the cases the bovine fetal membranes is shed within 6 to 8 hours after birth of the calf (van Werven et al. 1992). With the exception of the mare, domestic animals normally eat the afterbirths if they have access to them.

The nature of the third stage uterine contractions can be easily appreciated with the exercise of a little patience by direct palpation of the cow's uterus transrectally. At intervals of a few minutes, profound waves of contractions are generated, during which the texture of the uterus is transformed from a flaccid state into one of intense tone. Both the frequency and strength of uterine contractions significantly decrease by about 50% during the first 24 hours after calving (Bajcsy et al. 2005).

With the exception of the sow, the females of the other domestic species indulge in intensive licking of the newborn offspring. Within an hour of birth, it is normal for the young of all species to be suckling, and it is known that the stimulus of suckling causes release of oxytocin, which promotes the 'let down' of milk as well as an augmentation of myometrial contractions. This has been clearly demonstrated in the sow, in which suckling resulted in greater synchrony of the contractions and an increase in the number of tubocervical contractions (Ngiam 1977). Hence suckling exerts a favourable influence on expulsion of the afterbirth. In the mare, suckling appeared not to be significantly related to oxytocin release (Vivrette et al. 2000). Although there are no reports in the literatures of physiological measurements of postpartum uterine contractility in mares, it is most likely that the resumption of substantial contractions of the uterine musculature in the third stage cause abdominal pain, and it is quite common for expulsion of the membranes to be preceded by mild symptoms of colic.

Care of Parturient Animals

Mare

Mares approaching term should be put in a convenient paddock during the day and brought in at night. As soon as the udder and teats become distended or waxing occurs, the mare should be put in a foaling box at night and kept under continuous but unobtrusive observation. The majority of mares foal between 1800 hours and midnight, so that by daybreak in their natural wild environment, the foal has suckled and can gallop away. In this respect, it is noticeable that during the week before foaling myometrial EMG activity showed a progressive, reversible rise at night (McGlothlin et al. 2004). It is still uncertain to what extent foaling may be delayed as a result of some (to the mare) untoward environmental influence. There is experimental evidence showing that endogenous opioids suppress oxytocin release in pregnant mares, although this inhibition tended to decrease towards foaling (Aurich et al. 1996). When a continuous insensitive vigil is kept resulting in the mare being repeatedly disturbed, the mare seems as likely to foal by day as during the night. The cervix does not apparently require the same degree of preparatory changes as occur in the cow. A mare may even foal with ease soon after a gynaecological examination has been made when the cervix seemed relaxed but not at all dilated.

If the presentation is seen to be normal, i.e., two feet and muzzle at the vulva, then the mare is almost certain to deliver the foal; an exception is 'dog-sitting position' in which, with forelimbs and head showing, the presentation looks normal (see Chapters 11 and 14). As soon as faulty presentation, position, or posture is recognised (see Chapters 11 and 14) or if no progress occurs within 10 minutes of the onset of straining, a veterinary examination should be requested. In these ideal circumstances the obstetrician will have little more than an hour in which to arrive and deliver a live foal. The early dehiscence of the allantochorion in equine dystocia makes stillbirth the rule rather than the exception.

Cow

In order to follow the best management practices, cows and particularly heifers should be moved to a dedicated 'maternity unit' based on their 'due date' and/or signs of impending calving; namely complete relaxation of the posterior border of the sacrosciatic ligaments and a sudden enlargement of the udder. The accommodation should be clean, well-bedded, and the animals kept under frequent observation. If after 12 hours of restlessness there is no straining, a veterinary examination should be made to exclude primary uterine inertia, failure of the cervix to dilate, and/or uterine torsion (see Chapter 13). If a cow enters what appears to be a normal second stage and there is no progress after an hour's straining, she should be examined to ascertain the cause of the obstructive birth.

Ewe

Heavily pregnant ewes should be kept in a convenient paddock, lambing enclosure, or, preferably, a dedicated pen, where ewes can be kept isolated from the rest of the flock but with their own lambs. The location can be frequently changed during the lambing season to assist in infection control. Goats and sheep show a clear tendency to deliver more frequently during the day (Lickliter 1984, Fitzgerald & Jacobson 1992). However, constant obtrusive interference or farm management measures can override the natural tendency for parturition to occur during more quiet times.

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Goat

A similar system to that for ewes should be used, although frequently doe goats are kept in very small numbers and are treated individually.

Sow

This is discussed in Chapter 19. The majority of sows farrow during the evening and at night, when there are fewer disturbances in the farrowing barn.

Bitch and Queen Cat

A clean, comfortable, and warm bed in a quiet environment with minimal disturbance is preferable.

In all domestic species, the mother by reacting to the environment influences, can exert some control over the time of parturition. This is shown in a study involving five Beagle bitches in which it was observed that, it was observed that electromyographic activity of the uterus during late gestation was influenced significantly by external stimuli (van der Weyden et al. 1989). Parturient bitches that are transferred for whelping to a strange environment may suffer from nervous voluntary inhibition of labour. The maternal stress occasioned by the adverse surroundings is considered to inhibit the release of oxytocin and/or the resultant adrenaline secretion stimulates the ß-receptors of the myometrium, causing relaxation. The former mechanism has been very elegantly demonstrated in pigs (Lawrence et al. 1992).

Studies of 1151 calvings of beef cattle in Canada (Yarney et al. 1979) and of 522 parturitions in Friesian cows in Britain (Edwards 1979) showed that the distribution of calvings was fairly uniform throughout the 24-hour period but that the disturbances caused by farm staff at feeding and milking times exerted significant inhibiting effects, particularly in milking cows of third and later parities. When late pregnant dairy cows were offered silage in the evening, after a period of restricted access, a significant reduction of the incidence of night time calvings was observed (Gleeson et al. 2007).

There was also evidence from the beef cattle study and from data on lambing times collected by George (1969) that there can also be a genetic effect on the time of parturition; thus of the several beef breeds studied, 55.9% to 59% of cows with a Hereford grandparent calved during the day (between 0700 and 1900 hours), and under uniform husbandry, more Merino ewes lambed at night and more Dorset Horn ewes during the day.

The Process of Partuition

Mare

The imminence of labour can be recognised by the degree of mammary hypertrophy, waxing of the teats, and possibly the escape of milk from the glands (Fig. 6.10). Increasing calcium levels in mammary secretions indicate that foaling is impending (Cash et al. 1985). The best indication that the first stage has begun is the onset of patchy sweating behind the elbows and about the flanks. Although it occurs in the majority of mares, it is by no means invariable. It commences about 4 hours before the birth of the foal and increases as the stage progresses. Initially, the mare yawns, there are no obvious indications of pain, and food is generally taken readily. Respirations are normal and the pulse is about 60. (This increase in pulse rate is not significant of the onset of labour for it develops during the terminal stages of pregnancy.) There is evidence that body temperature may become slightly subnormal during the first stage (36.5°–37°C), but using this to predict the onset of foaling is unreliable.

• **Fig. 6.10** Mammary hypertrophy, tumefaction of the teats, and waxing in a thoroughbred mare 4 hours before the birth of the foal.

As the stage advances the mare becomes restless and tends to wander aimlessly around the loose box. The tail is frequently raised or held to one side. There may be swishing of the tail or slapping of it against the anus and kicking at the abdomen. As the end of the stage approaches the mare becomes very restless. This is indicated by crouching, straddling of the hindlimbs, going down on the knees or sternum and rising again, glancing at the flank. The stage terminates with rupture of the allantochorionic membrane and the escape of urine-like, allantoic fluid from the vulva. Its quantity is not copious. It will be noticed that there is no reference to visible straining during this period in the mare.

The onset of the second stage occurs abruptly. It is characterised by the appearance of the amnion or the commencement of forcible straining. There is never very much delay between them, and they often coincide. Very soon after straining begins, the mare goes down. She passes on to her side with limbs extended and generally remains in this position until the foal is born. The presence of the transparent bluish-white water bag (amnion) at the vulva is quickly followed by the appearance in it of a digit. Straining efforts recur at fairly regular intervals; each bout comprises three or four powerful expulsive efforts followed by a period of rest, generally of about 3 minutes. When the mare rises during this stage, the foal may partly slip back into the abdominal cavity. During expulsion one forelimb precedes the other by a distance of about 7 to 8 cm and this position is maintained until the head is born. The point is a significant one, for it indicates that one elbow passes through the bony pelvic inlet before the other and in this way nature has provided for the foal to present the minimum obstruction at the pelvic inlet. During its delivery, the head is generally in the oblique position; it may even be transverse—the cheek lying on the limbs—but this is probably due to rotation of the cervical joints within the pelvis and should not be taken as evidence that the presentation was oblique. The second stage is illustrated in Fig. 6.11.

The greatest and longest effort is associated with birth of the head; the chest presents less difficulty, and after this the hips slip

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out easily. Although equine delivery is comparatively rapid, it constitutes a tremendous effort, and after expulsion of the foal the mare may remain lying on her side exhausted for anything up to 30 minutes.

The umbilical cord is intact when the foal is born. It subsequently ruptures, 5 to 8 cm beneath the belly, at a point where there is a sphincter of smooth cells lying beneath the amniotic covering, as the result of movement by either the mare or the foal. Usually also, the foal is born within the amnion, the membrane being ruptured by the movements of the foreparts of the fetus. Respiratory movements may be seen within the intact amnion. The lower portions of the foal's hindlimbs often remain within the mare's vagina for some minutes after the rest of the foal is born. Their final emergence is due to movement of the foal rather than to the expulsive efforts of the mare.

The duration of the second stage in the mare is about 17 minutes; it may be as short as 10 minutes. The longest the writer has observed within normality was 70 minutes, and in this case, it was seen that the greater part of the placenta came away with the fetus. It is probable that this is near the limit of the time available if parturition is to remain normal, for in the mare separation of the placenta tends to proceed rapidly once the second stage begins,

• **Fig. 6.11** The second stage of labour in the mare. Note the relative positions of the forelimbs and head. The foal is born in the amnion but breaks out of the sac without difficulty. In the final photograph much of the allantochorion has been passed but the umbilical cord is still intact. *Continued*

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• **Fig. 6.11, cont'd**

and if delivery occupies too long a time it is likely the fetus will succumb from this cause.

In the majority of mares the membranes are expelled quickly after the birth of the foal, generally within 3 hours; in fact, they may fall away in about 30 minutes. The average duration of the third stage is about 1 hour (Steven 1982). Occasionally, however, cases are met in which periods up to 24 hours elapse before the membranes fall away, yet the animal suffers no ill effect. Straining is not a feature of the third stage, the afterbirth being expelled by the myometrial contractions. The recognition of the exact time at which a case becomes pathological and interference is necessary is difficult (see Chapter 32). The definition of when fetal membranes are considered to be retained in the horse is not consistent in the published literature (Sevinga et al. 2004). As has already been pointed out, the membranes are passed with the allantoic surface (smooth and shiny) of the allantochorion outermost. This statement holds for those cases in which expulsion occurs early after parturition, but it has been noticed that in those in which there is delay, the fetal placental surface (roughened and red) is outermost, indicating that separation was complete before expulsion began.

Cow

There are many signs of impending parturition (also called *prodromi*), but the question is which ones are the most useful in the management of calving. Swelling of the vulva, discharge of mucus,

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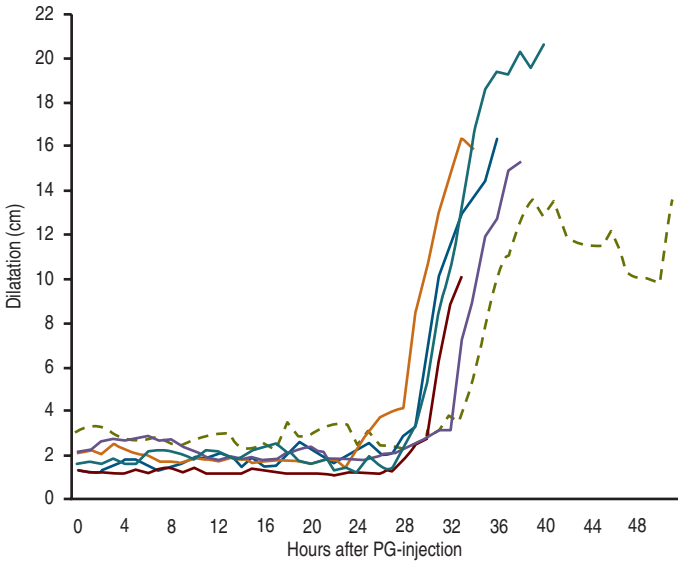
changes of the udder, and relaxation of the pelvic ligaments have been studied in detail in relation to the time interval to the moment of birth of the calf (Berglund et al. 1987). However, given the rather subjective nature of these parameters and the wide range of the intervals found in such studies, these prodromi are not very helpful in making an accurate prediction.

About 54 hours before birth of the calf, Ewbank (1963) noticed a fall in the cow's body temperature of 0.6°C. He observed that a cow showing signs of imminent labour would be unlikely to calve during the succeeding 12 hours if its temperature was 39°C or more. Parturition will usually begin within 12 hours of the appreciation of complete relaxation of the posterior borders of the sacrosciatic ligaments. Changes in vaginal temperatures have been used to predict parturition in beef cows with single and twin pregnancies (Aoki et al. 2005). Behavioural characteristics, such as the recording the time and duration of when and for how long the cow is standing or recumbent can be useful, provided it is done remotely and the animal is not disturbed. On the last day before calving, cows spend a significantly greater number of periods in a standing position (Huzzey et al. 2005). In a recent study from Hungary (Kovács et al. 2015), changes in maternal heart rate (HR) and heart rate variability (HRV) were recorded around unassisted calvings on a large dairy farm. It was concluded that changes in HRV are more immediate predictors of the onset of calving than behaviour or HR, as they occurred before the onset of calving restlessness. So it can be anticipated that, with the further development of automatic recording devices in farm animals, fairly accurate prediction of calving time will become one of the possible applications.

The first stage of labour can easily be recognised by direct palpation of the cervix. There is great variation in the intensity of the symptoms of the first stage; in fact, many subjects, particularly multigravida, show none. Others, usually heifers, may show signs of abdominal pain for periods up to 24 hours before the cervix is completely dilated. The first stage usually lasts some 6 hours. By suturing two ultrasonic crystals (one transmitting, the other receiving pulses) opposite to each on the caudal cervical rim of the cervix, continuous changes in the distance between these crystals has been used to assess dilatation during induced calvings (Breeveld-Dwarkasing et al. 2002). By combining this technique, with in vivo electromyography of the myometrium, the relationship between dilatation, uterine activity, and hormonal changes was examined during induced calvings (Breeveld-Dwarkasing et al. 2003). Fig. 6.12 demonstrates that after a single injection with $PGF_{2\alpha}$ on day 274 of gestation, the increase of cervical diameter follows a rather consistent pattern. On average the cervix started to dilate 28.5 hours after prostaglandin injection. From this time point on, uterine diameter increased linearly at an average rate of 1.6 cm per hour in the calvings in which the calf is in anterior presentation. The mean interval between onset of dilatation and birth of the calf (when the crystals were removed again from the cervix) was 9.3 hours.

Another feature of the cow is that occasional straining may occur during the first stage. Food is only 'picked'; rumination is irregular; there may be 'lowing' or kicking at the belly. The animal is obviously restless; she may stand with her back arched and tail raised; she may go down and rise again frequently. The line of demarcation between the first and second stages is therefore not always clear-cut, as it is in the mare. Body temperature is generally normal, but the pulse rate is often increased to between 80 and 90. In 40% of normal calvings observed by the authors, the intact allantochorion reached the vulva as a 'first water bag'.



• **Fig. 6.12** Dilatation curves of the caudal part of the cervix of 5 cows with calves in anterior presentation (straight lines) and of one cow with her calf in posterior presentation during the hours after a single injection with $PGF_{2\alpha}$. (From Breeveld-Dwarkasing et al. 2003a.)

The second stage is less intense but of longer duration than in the mare. Straining is less frequent, and the animal often remains standing at first (Fig. 6.13). During the passage of the head through the vulva, however, the cow generally goes down and remains recumbent until the calf is born. She may lie on her side but more often adopts sternal recumbency. Detailed observations of 463 calvings in a dairy herd revealed some interesting data on the duration of this expulsive stage in relation to the survival of the calves (Gundelach et al. 2009). They defined the second stage of labour (SSL) as the interval from rupture of the allantoic/amniotic sac to passage of the forehead or pelvis of the calf through the vulva (Table 6.1).

In twin births, Owens and colleagues (1984) noted that intense straining for the birth of the second calf began 10 minutes after the delivery of the first calf. During the second stage, temperature may rise to 39.5° or 40°C, but this is by no means constant and is probably dependent on the degree of effort required. The pulse rate may increase to 100 or more. About 20% of calves are born almost completely enclosed in the amnion.

Placental separation occurs more slowly in the cow than in the mare, and thus the stage of expulsion may be considerably longer without jeopardising the life of the young one. The process of expulsion is similar to that described for the mare. The umbilical cord is shorter in the calf than in the foal, and its rupture generally occurs as it falls from the vulva.

Expulsion of the fetal membranes usually takes place within 6 hours after calf expulsion (Van Werven et al. 1992); occasionally, it may be delayed to 12 hours, but when 24 hours elapse and the membranes are still in the uterus, it is probable that the cause is pathological. Unless prevented from doing so, it is customary for the cow to eat the fetal membranes. It will also be noticed during the first and second stages there is a tendency to lick up vulval discharges.

Ewe and Doe Goat

In the ewe the course of parturition is fairly similar to that described for the cow, except that the incidence of twinning and even triplets

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• **Fig. 6.13** The second stage of labour in the cow. Note the relative positions of the fetal head and forelimbs. The allantoic and amniotic sacs can be clearly distinguished in (C).

is high in those ewes that have been flushed before the mating time or in those breeds with high fecundity. Increased restlessness, walking (in circles), isolation seeking, and vocalising are the most prominent signs of impending lambing (Arnold & Morgan 1975). Australian authors Arnold and Morgan reported that most ewes had a labour of less than 1 hour with very few more than 2 hours. Length of labour for second born lambs was significantly shorter than that for first born twins. The interval between the birth of the first and second lamb of a twin pair was most commonly between 11 and 20 minutes and generally less than 45 minutes. Factors affecting this interval have not been systematically investigated. However, in ewes with bilaterally disposed twins, sequential radiographs have shown that both fetuses assume the normal disposition for birth more or less synchronously, but subsequently only one of them will engage the birth canal; in the other the postural changes regress so that the carpal joints are no longer extended, and the head returns towards a vertex presentation from a facial one (Fraser & Terhurne 1977). Which of the two fetuses is destined to be expelled first seems to be regulated by the degree of contractility of the two uterine horns. Once pressure waves have become established in the parturient ewe, one horn develops contractions of about double the amplitude of those in the other horn but with no difference in the frequency; as a consequence, it is the dominant horn that subsequently expels the first fetus (Hindson et al. 1968). Although this study has never been confirmed by others, further experimental evidence suggests that functional differences

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TABLE 6.1 The duration of second stage labour in dairy heifers and cows in relation to the occurrence of stillborn calves (classified as being dead at birth or failed to survive for 24 hours after birth)

Mean (± SD) Duration of Second Stage Labour	
Heifers with live calves:	135 ± 86 min
Heifers with stillborn calves:	250 ± 120 min
Cows with live calves:	77 ± 68 min
Cows with stillborn calves:	156 ± 100 min

From Gundelach et al. 2009.

between the two uterine horns at parturition may exist. When premature parturition was induced in bilaterally pregnant sheep with ACTH infusion of only one fetus, it was the infused fetus which was expelled first (Liggins et al. 1977).

Some 94% to 97% of lambs are born in anterior presentation (Naaktgeboren & Slijper 1970), and spontaneous birth may occur despite retention of a forelimb. Ewes expel the afterbirth within 2 or 3 hours of the expulsion of the lamb.

Observations of parturitions in goats (Beetals and crossbreds with Alpine and Saanen; Das & Tomer 1997) revealed that more than 90% of the kids are born in anterior presentation. The average duration of expulsion (interval between appearance of the allantoic sac and complete expulsion of the fetus) was 20 minutes for the first kid and 6 minutes for the second one. Male kids took some 20% to 35% longer to be expelled. On average the fetal membranes were expelled 2.5 hours after birth of the (last) kid.

Sow

Sixty to 75% of sows farrow at night (Bichard et al. 1976, Kovenic & Avakumovic 1978). The fetal membranes of adjacent piglets are usually fused (see Chapter 4) and because individual or aggregated afterbirths may be expelled during the phase of fetal expulsion as well as after the birth of the last fetus, it is unrealistic to speak of separate second and third stages of labour in porcine species. Good descriptions and quantitative data of normal farrowing have been given by Jones (1966), Randall (1972), and van Dijk and colleagues (2005).

Sows in late pregnancy are quiet resting and frequently sleeping in lateral recumbency, but within 24 hours before the birth of the first piglet, a marked restlessness develops, accompanied by bed-making activity (if they have access to suitable materials). Endogenous $PGF_{2\alpha}$ appears to be involved early in an endocrine pathway that involves the brain and is responsible for most of the prepartum behavioural components (Gilbert 2001). The intensely active period is followed by recumbency and rest. The onset of the parturient pattern of myometrial activity occurs between 6 and 9 hours before the first piglet is born (Taverne et al. 1979a). There are several alternating periods of rest and restlessness, and then, in the hour preceding the birth of the first piglet, the sow settles quietly into lateral recumbency. Conspicuous mammary growth is a feature of late gestation; 1 to 2 days before farrowing the individual glands are clearly demarcated, turgid, tense, and warm, and milk can be expressed from the prominent teats during the final 12 to 24 hours. There is progressive swelling of the vulval labia from about 4 days before parturition and the mucosa becomes reddened. Prepartum temperature variations of between 37.5° and 38°C, but with no constant temperature change, have previously been reported, but Elmore and associates (1979) recorded a 1°C rise at 12 to 15 hours before the birth of the first piglet.

Parturient sows usually remain in lateral recumbency, but gilts more particularly may stand after the birth of the first or second piglet or change from one side to the other or from lateral to ventral recumbency. After the prepartum quiet period, there is intermittent straining accompanied by paddling leg movements. The birth of the first pig (and subsequent ones) is heralded by the passing of a small quantity of fetal fluid and by marked tail-switching. The greatest parturient effort is expended over the first piglet, with succeeding fetuses being expelled with surprising ease and sometimes with projectile force. The allantochorion and amnion usually rupture as the conceptus traverses the birth canal, but occasionally piglets are born within the amnion, and, not uncommonly, a fetus becomes surrounded by the membranes of another fetus. Only small amounts of fetal fluid are voided.

The expulsive phase of parturition is illustrated in sequence by Fig. 6.14. By means of laparotomy and transuterine marking of the uterine location of fetuses of the miniature pig between 80 and 105 days of gestation and then observing their birth sequence, Taverne and colleagues (1977) found that the offspring were delivered randomly from both uterine horns. As had previously been observed by Perry (1954), there were occasional instances of a piglet apparently 'overtaking' its neighbour in the uterus. In 18 of 95 piglets the observed presentation at birth differed from that previously detected at laparotomy, but it was not determined whether these changes of polarity occurred in the uterus during gestation or within the uterine body during delivery. Both explanations would seem valid, although we postulate that the polarity change is most likely to occur because a piglet passes down from one horn into the base of the other and is then expelled into the uterine body and then the outside. Observing the birth of 1078 piglets in 103 litters Randall (1972) recorded 55.4% anterior and 44.6% posterior presentations. In both presentations the fetus was usually in dorsal position with the presenting limbs flexed alongside the fetal body; thus the fetal snout or tail was the first part to protrude. The mean interval between consecutive births was 16 minutes, and the mean duration of the expulsive stage was 2 hours 36 minutes. In the analysis of data from some 211 litters of several breeds, van Dijk and associates (2005) found that breed, litter size, and gestation length significantly affected the duration of the expulsive stage. A curvilinear relationship between birth interval and rank (relative position in the birth order) of the piglets was found; piglet birth intervals increased with an increasing birth weight, and both stillborn and posteriorly presented piglets were delivered after significantly longer birth intervals than live born and anteriorly presented piglets. Between 60% and 70% of piglets were born with intact umbilical cords; in the case of the early born fetuses the elasticity of the cord allowed the newborn piglet to reach the middle of the sow's abdomen without the cord breaking. The later born piglets were more likely to be expelled with broken cords (Randall 1972). Newborn piglets are usually remarkably active and may reach a teat within 30 minutes after expulsion and attempt to suck, although this largely depend on their viability and floor of the farrowing crate.

The porcine fetal membranes tend to be expelled as two or three masses of joined allantochorions, with the placental stalks of the umbilical cords indicating the number of separate conceptuses in each mass; single afterbirths may also be voided. One or more of the coalesced masses are commonly expelled before all the fetuses

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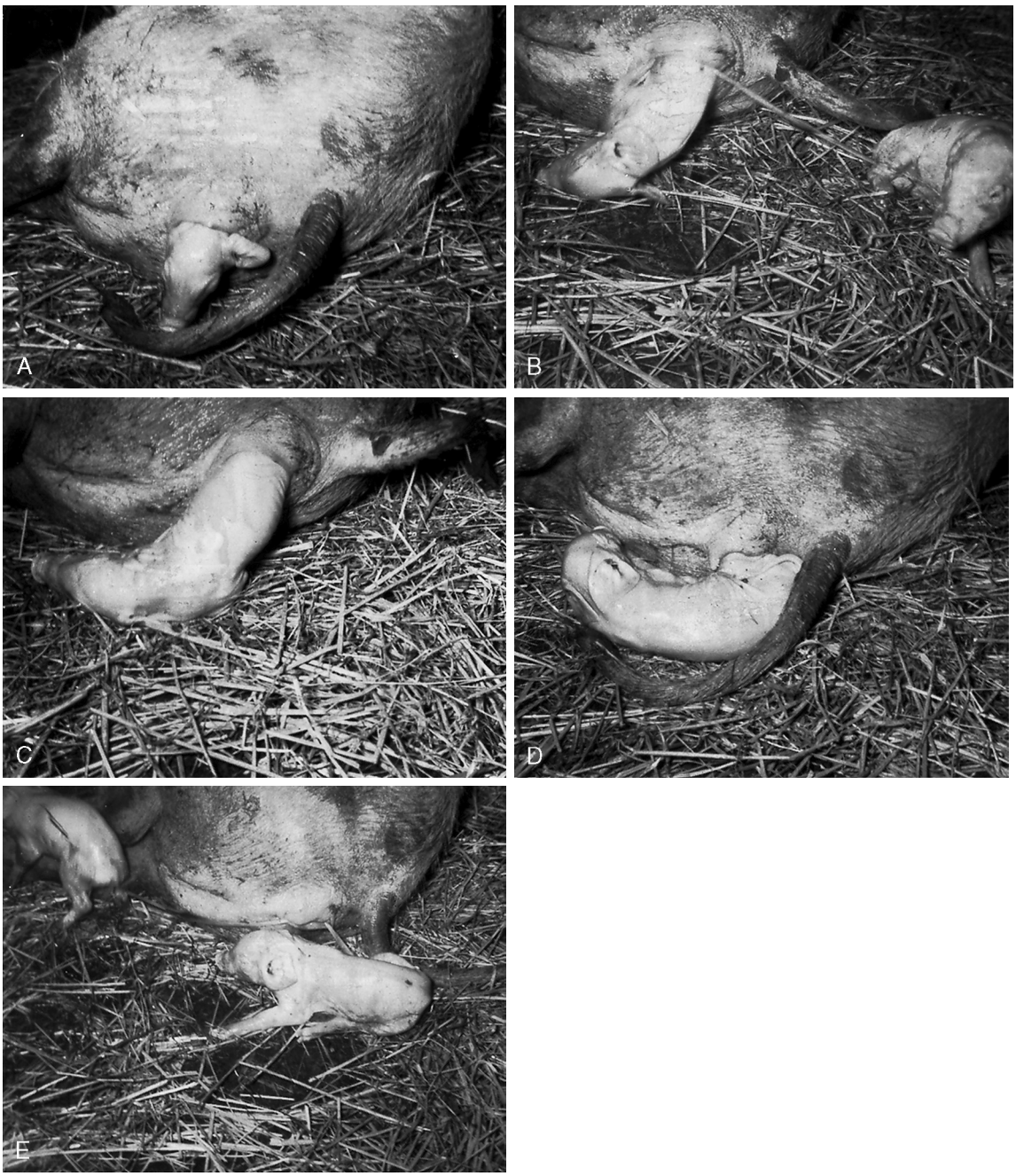

• **Fig. 6.14** The second stage of labour in the sow. Note the lack of extension of the forelimbs. The intact umbilical cord can be seen in (E).

are born, but the largest mass is usually expelled about 4 to 5 hours after the last piglet; prolonged parturition impairs placenta expulsion (Bjőrkman et al. 2017). When all the piglets have been expelled, the sow usually stands up and micturates profusely. She then lies down again, sometimes very clumsily—with consequent risk of crushing the surrounding piglets; the feature of the next phase is that the sow lies quietly for a long time and allows the piglets to suck.

A notable economic feature of porcine parturition is the frequency of stillbirths. They occur in about 30% of apparently normal farrowings, and the overall stillbirth percentage in unassisted deliveries is 3% to 6% (Randall & Penny 1970, Leenhouwers et al. 1999). Because piglets are nearly always found alive when premeditated hysterectomies or hysterotomies are performed just before the onset of parturition, it is concluded that stillbirths occur during farrowing. Early born piglets are more likely to survive than either the middle born ones or the late born ones from the tips of the uterine horns (Dziuk & Harmon 1969, Sprecher et al. 1974, Leman et al. 1979). The stillbirth rate is also influenced by litter size, being greatest in litters of four or less or in those of 14 or more, and by the polarity of the expelled fetus (Sovjanski et al. 1972, van Dijk et al. 2005). Piglets at the ovarian ends of the uterine horns have to traverse the entire length of the respective horn and, in the case of small litters, they may have to negotiate a previously unoccupied and nondilated length of horn. Piglets born in posterior presentation are nearly four times as likely to

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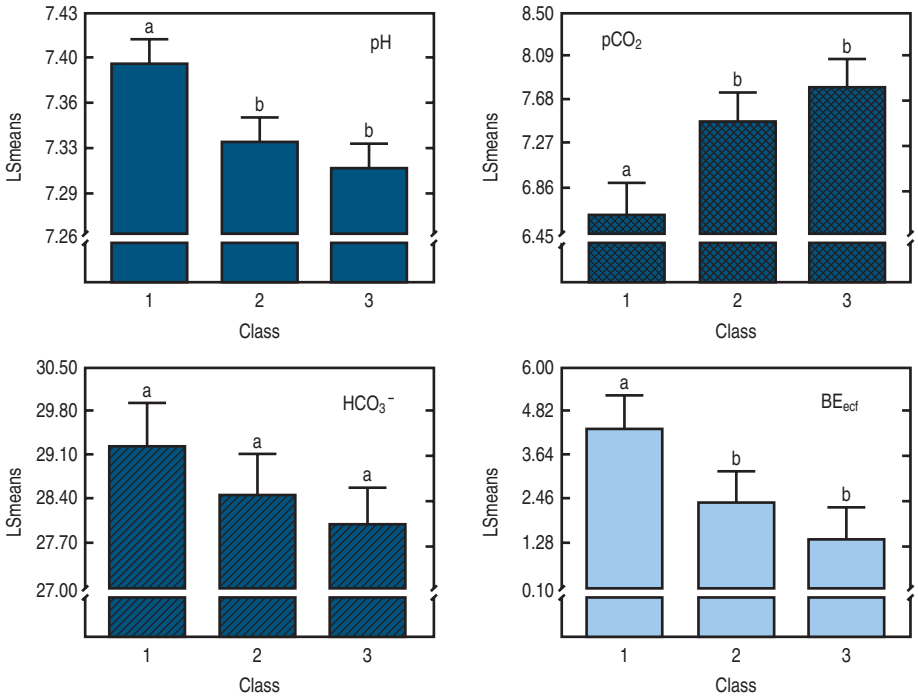


• **Fig. 6.15** Acid–base values in blood samples taken from the cord of liveborn piglets, delivered during the first, second, and last third part of the litter (class 1, rank ≤ 0.33; class 2, 0.33 < rank ≤ 0.66; class 3, 0.66 < rank ≤ 1). (From van Dijk et al 2006.)

be born dead as those presented anteriorly; also piglets born alive in posterior presentation or expelled during the final third of the litter show significantly worse acid–base balance values in blood samples taken from the cord at birth (Fig. 6.15; van Dijk et al. 2006). As already mentioned previously, extension of the intervals between successive births of up to 20 minutes or more also predisposes to stillbirth.

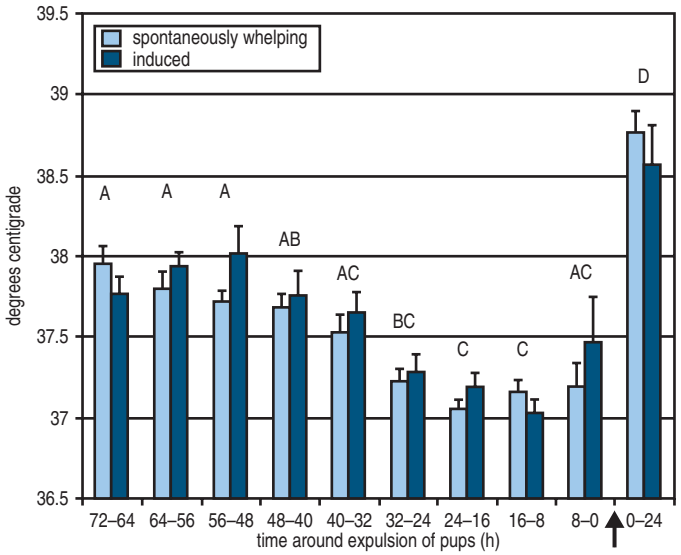
High progesterone and/or low oestrogen levels, produced experimentally in the preparturient sow's blood, can delay farrowing and increase the stillbirth rate from 10% to 97% (Wilson et al. 1979). It is not known if such hormonal derangements also occur naturally.

Bitch

The imminence of parturition is indicated by the animal preparing her bed and becoming restless. In primigravida the onset of lactation more or less coincides with parturition, but in multigravida milk may be expressed from the teats for several days before its onset. In most bitches there is a transient fall in body temperature of at least 1.2°C within the 24 hours before the onset of labour, but it needs repeated measurements to detect a temporary lower value. The prepartum temperature drop also occurred when whelping was induced (Fig. 6.16; Baan et al. 2005).

There is nothing characteristic about the first stage, but it is generally noticed that the bitch is restless, indifferent to food, and inclined to pant. It is most obvious in primigravida and occupies about 12 hours.

Electromyographic (EMG) pattern during late pregnancy comprises episodes of myoelectrical activity (EMEAs) lasting 3 to 10 minutes and recurring at a low frequency (maximum 2.5/hour). During the last 7 days before whelping and especially during the last 48 hours, more short bursts (<3 minutes) appeared between the EMEAs; this was closely correlated with the decline in progesterone concentrations. The total duration of EMG and the burst



• **Fig. 6.16** Rectal temperatures measured every 8 hours from 64 to 72 hours before expulsion of the first pup *(arrow)* until 0 to 24 hours after expulsion of the last pup in 6 spontaneously whelping dogs *(light blue bars)* and in 6 dogs in which parturition was induced with the progesterone-blocking agent aglepristone. (From Baan et al. 2005.)

frequency increased dramatically as the progesterone concentration and body temperature fell 12 to 24 hours before whelping (van der Weyden et al. 1989). Bitches apparently are aware of the uterine contractions at this stage, because they frequently stand or change their position but remaining recumbent when contractions occur. The onset of the second stage is indicated by abdominal straining, but at first this may be difficult to recognise because

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of the positional changes of the bitch. In the majority of cases the animal remains in her bed in sternal recumbency, although sometimes she may stand and move about during straining efforts. The allantochorionic membrane of the first fetus appears at the vulva, and after a series of expulsive efforts attains the size of a golf ball. It is generally ruptured by the bitch, who licks vigorously at her vulva. As with other species, delivery of the head coincides with the greatest effort; in the majority of instances, once this is born the remainder of the fetus follows easily. Expulsion of the first fetus, as judged by the duration of abdominal straining, may occupy up to an hour, but seldom longer if the process is normal. It is often quicker, a matter of a quarter of an hour or so. Although some 40% of all puppies are born in posterior presentation, repeated measurements of acid–base balance in jugular blood samples after birth showed that pups born in posterior presentation experienced a more severe metabolic acidosis (van der Weijden et al. 1989).

The umbilical cord is intact at the birth of the puppy, and occasionally, they can be seen attached to their cord when the mother rises immediately after expulsion. The cord is either quickly torn or the bitch bites it when eating the placenta. Normally, the bitch rests for a time after the birth of her first puppy. She lies licking it, and soon it begins to move and tries to suckle when it is lively enough to reach a nipple. She pays frequent attention to her vulva and licks up any discharges. The placenta is generally voided in 10 to 15 minutes (or even together with the pup) and is promptly eaten by the bitch.

Straining recommences after a variable delay. This delay is usually less than 30 minutes, but it may frequently be as long as 1 to 2 hours; the author has seen it occupy 7 hours in a bitch pregnant with two fetuses only. The effort required and time occupied for the delivery of the second fetus is usually less than for the first, but this is mainly dependent on the presentation, position, and weight of the pup. The pattern of expulsion of the fetuses is most irregular; one bitch may have her first puppy, then rest for several hours, then deliver two or three more in quick succession, and then rest again before expelling several more. Another may expel them at fairly regular intervals throughout the period. Mean intervals between the births of two successive puppies ranged between 30 and 60 minutes in most breeds (Naaktgeboren et al. 2015). In exceptional cases a bitch may deliver the whole of her litter in an hour or so. In a study involving spontaneous whelping in Beagle bitches, the mean interpuppy interval was 1.9 ± 08 hours (Baan et al. 2005); however the progress may have been influenced by the experimental protocol involved in the study with blood sampling and other interventions. Parturient bitches are indeed very sensitive to environmental disturbances, and stress may easily cause a delay of the expulsion stage, probably by elevating maternal catecholamines and decreasing oxytocin levels (Naaktgeboren et al. 2015). Plasma oxytocin levels are elevated during the expulsion stage (Olsson et al. 2003), and there are temporary elevations, although not exclusively, around the time of expulsion of a pup (Klarenbeek et al. 2007). In bitches with primary uterine inertia, plasma oxytocin levels were found to be significantly lower than in normal whelping controls (Bergström et al. 2006). Unlike the sow (see earlier in this chapter), there is a significant tendency for the puppies to be expelled from alternate horns (van der Weijden et al. 1981). In addition, reversal of the presentation or 'leapfrogging' of puppies is very uncommon.

Expulsion of the placentae is also irregular. They may come individually (immediately) after each pup. In other instances a puppy may be born with the membranes of its predecessor around its neck, indicating that this pup has passed its predecessor's placental girdle when still attached to the uterine wall. Exceptionally, there is a delay of up to 24 hours before all placentas are expelled and parturition is finally completed.

The total time occupied by the second stage will depend chiefly on the number of fetuses in the litter, but as a general rule, when the litter is within the usual limits (four to eight) it occupies some 4 to 8 hours. The question arises over what is the maximum normal time it may occupy, especially when the number of fetuses is very high (10–14); Naaktgeboren and colleagues (2015) reported up to 13 hours for the largest breeds with the largest litters. It is very improbable that puppies born after this time, even without assistance, will be alive, although the birth of a live pup has been reported even after an interval of 34 hours (Romagnoli et al. 2004). Compared with other domestic species, stillbirth rate in dogs appears relatively high. In a study from Norway, stillbirths and deaths within the first week were responsible for the majority of puppy losses (Indebrø et al. 2007). A stillbirth rate of 10.9% in 98 litters from four different large breeds occurred, and litter size significantly affected stillbirth rate. A previous study of Boxers (a brachycephalic breed) in The Netherlands (Nielen et al. 1998) reported that in a group of 571 bitches, deaths between birth and day 49 postpartum, 25.7% were stillborn. In contrast, a much lower stillbirth rate was reported more recently in a large cohort study involving 224 breeds involving data from the time of birth for all purebred litters registered by the Norwegian Kennel Club (Tønnessen et al. 2012). Perinatal mortality was defined as the sum of stillborn puppies (4.3%) and puppies that died before 8 days after birth (3.7%); it was present on 24.6% of the litters. However, the variable sources of the original whelping data (individual breeders, research, and commercial breeding colonies) may be responsible for the large variation in perinatal losses.

A feature of parturition in the bitch is that much of the uterine discharge is dark green in colour. This is due to the breakdown of the marginal haematoma ('green border') and to the escape of the blood pigment, biliverdin or uteroverdin.

Queen Cat

During the last week of pregnancy, the queen will seek out a suitable nesting area for kittening. Most cats are secretive about kittening and will select a quiet, undisturbed spot, but some socialised pet cats show less interest in selecting a suitable nesting position and become more demanding of human contact.

Mammary development becomes noticeable in the last week of pregnancy, and this may be particularly prominent in maiden queens. Unlike in bitches, rectal temperature does not fall before the onset of parturition.

During the first stage of parturition some queens may become restless, frequently visiting the site selected for kittening, whereas other queens hide away quietly in the chosen nesting area, occasionally lying down and straining unproductively. Frequent licking of the vulval area and panting are other signs that parturition has started. The second stage begins with straining, with the queen usually in lateral or ventral recumbency or in a defecating position. There are only few published data on the course of parturition in cats, and the ones now available are based on questionnaires returned by individual breeders. Sparkes and colleagues (2006) reported a median interval, between the first observed contractions and birth of the first kitten, of 0.5 to 1 hour and with more than 80% of the queens taking less than 2 hours. The duration of the entire expulsive stage (interval between first and last kitten; n = 858) also varied considerably, but in some 85.7% of the animals, it took less than

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6 hours, and in three queens more than 48 hours elapsed to the completion of expulsion. The interval between individual kittens within a litter (n = 1001) also varied; in 73.6% of the recorded events (n = 1001), it was less than 1 hour and in 21.1% between 1 and 3 hours. Another study (Musters et al. 2011) reported a median interkitten time of 30 minutes (range 2–343 minutes), with 95% of the kittens born within 100 min after the preceding kitten. The latter study also found that interkitten time was not influenced by the kitten's gender, presentation at expulsion, birth weight, stillbirth, or parity of the queen. Sixty-nine percent of the kittens were born in anterior presentation and 31% in posterior presentation, indicating that both presentations can be considered as normal. A stillbirth rate of 7.2% was reported by Sparkes and associates (2006), whereas in the study of Musters and colleagues (2011), some 5% of the kittens were born dead.

The placentas with fetal membranes are usually expelled still attached to the fetuses or separately shortly afterwards. The mother vigorously licks the newborn kitten and quickly consumes the placenta, thereby severing the umbilical cord. It may take some 30 to 40 minutes after birth for the kittens to reach a nipple and start sucking.

Care of the Newborn

Readers of this section of the chapter might wish to consult Mee (2008) for an authoritative description of the management of the newborn calf. Much is applicable to all domestic species. The sudden change at birth from the constant, controlled, cosseted environment of the uterus to the variable and frequently stressful free-living environment demands great adaptability from the newborn. In domestic species, however, provided that parturition is normal, most survive this transition without assistance. It should also be remembered that during the latter part of gestation the fetus is already undergoing a number of maturational changes, probably stimulated by the hormonal changes that occur in the initiation of parturition, in preparation for the free-living state. However, there is often a high mortality rate more often associated with dystocia; in cattle, for example, it has been estimated that 64% of calf losses occur within 96 hours of birth (Patterson et al. 1987), and those born after dystocia are 2.4 times more likely to develop infectious diseases within the first 45 days of life than those born as a result of a normal birth (Toombs et al. 1994). A data set of Holstein calving records, comprising 4103 herds with 2,304,278 calving events representing 1,164,233 cows and 96,069 twin births, reported twinning trends and calf mortality across time (Silva del Rio et al. 2007). Overall, the reported twinning rate was 4.2%; calf mortality was greater after twin births, with 28.2% of twin calving events reporting one or both calves as dead, compared with 7.2% for singleton births (OR = 6.5). Calf mortality for primiparous and multiparous cows was 5.0% after a single birth and 25.5% after twin births, whereas for nulliparous heifers, mortality was 10.4% for singletons and 38% for twins (OR = 3.4). Although these data refer to calvings, it clearly shows the need for increased surveillance of primigravid animals of all species. In dogs a large survey in France involving 27,221 bitches and 37,946 litters recorded a stillbirth rate of 0.4% and a combined stillbirth and perinatal mortality rate of 13.4% (Chastant-Maillard et al. 2017). In a similar survey in cats involving 5303 queens from 45 breeds, the stillbirth rate involving 28,065 kittens was 8.5% (Fournier et al. 2017). At birth and for a variable period of time afterwards, a number of important events must occur, and it is the responsibility of the person involved in supervising or assisting at the parturition to assist the newborn so that the likelihood of survival is enhanced.

Recently, in the bitch, attempts have been made to utilise some of the methods that have been used in human obstetrics to monitor the dam and the new born puppies. In human obstetrics/neonatal paediatrics, the APGAR system has been used to assess the newborn baby; APGAR is an acronym derived from: Appearance, Pulse, Grimace, Activity, Respiration. Although this is not directly applicable to veterinary medicine, a comparable scoring system based on the following has been evaluated, namely assessment and scoring of: heart rate, respiration, irritability reflex, motility, and membrane mucus colour (Veronesi 2016). Similarly, Groppetti and associates (2010) not only evaluated the modified APGAR system, but also assessed puppy acidosis by measuring umbilical vein lactate concentrations and myometrial activity using a commercially available tocodynamometric system.

Onset of Spontaneous Respiration

During fetal life, episodes of muscular movements similar to those of respiration have been observed in a number of species; whether these are truly the precursors of the continuous respiratory movements of the newborn is debatable. However, if parturition occurs normally, then spontaneous respiratory movements will occur within 60 seconds of expulsion; if there is a delay, then respiratory movements can sometimes occur before the offspring has been completely expelled.

There are probably a number of factors that are responsible for the initiation of spontaneous respiration. During the birth process, P_{O_2} and blood pH are falling and P_{CO_2} is rising due to the start of placental separation and occlusion of the umbilicus, thus restricting gaseous exchange. These changes have been shown in the lamb to stimulate chemoreceptors in the carotid sinus (Chernick et al. 1969). Tactile and thermal stimuli are also important, for it has been shown that if the face of the fetal lamb is cooled, there is stimulation of respiratory movements (Dawes 1968); the licking and nuzzling of the dam probably provides some stimulus.

The first respiratory movement is usually a deep, forceful inspiration that is necessary to force air into the lungs. It has been demonstrated in the fetal lamb that a pressure of 18 cm of water is necessary to force air into the lungs at the first inflation; once this occurs, only a small pressure is needed to cause full inflation thereafter (Reynolds & Strang 1966). Pulmonary surfactant, produced by type 2 pneumocytes during the maturation of the fetus at the end of gestation, assists in the initial lung expansion and alveolar stabilisation. Although the initial work done in the first breath is greater in mature than in immature fetal lungs, for the second and subsequent breaths it is much less; this is because the alveoli remain partially inflated after exhalation. As a result of lung inflation, the pulmonary vascular bed opens up followed by a sudden increase in pulmonary blood flow. Such changes in the vascular dynamics result in the rapid closure of the ductus arteriosus and foramen ovale, followed by the ductus venosus several hours later; the consequence of these changes are that instead of gaseous exchange occurring via the placenta, it now occurs via the lungs (Grove-White 2000). Survival of the newborn is dependent on the rapid onset of normal, spontaneous respiration. In a normal calf, respiration usually commences after 30 seconds of birth, being irregular at first before settling down to 45 to 60 breaths per minute (Grove-White 2000). Once birth is complete, it is important first to ensure that the upper respiratory tract is cleared of fluid, mucus, and attached fetal membrane. This

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TABLE 6.2 Equipment for resuscitation of foals

Endotracheal tubes 7–12 mm diameter
Oxygen cylinder and regulator
Resuscitator bag
Doxapram
Adrenaline (epinephrine)
Sodium bicarbonate 4.2%
Dextrose saline 5%
Valium
Dexamethasone phosphate
Lidocaine
Intravenous catheters
Drip sets
Enteral feeding tubes
Needles, syringes, blood collection tubes
Glucose meter

From McGladdery 2001.

can be done with the aid of fingers or, preferably, with a simple suction device. Elevation of the rear of the calf, particularly by suspension from the hindlimbs, results in the escape of copious quantities of fluid. Some of this comes from the stomach, and it may not necessarily be beneficial because it has been shown that one-third of this fluid can be absorbed from the lungs of the newborn via the lymphatic system (Humphreys et al. 1967). In addition, pressure on the diaphragm by the abdominal viscera can itself interfere with normal respiratory movements. Brisk rubbing of the chest with straw or towels frequently provides the necessary tactile stimulus to stimulate respiration, although a portable oxygen cylinder and resuscitator are useful pieces of equipment to have available. They comprise a small portable cylinder of oxygen, a reducing valve, a rebreathing bag, and either a face mask or an intranasal or endotracheal tube; the latter is preferable because, unless the oesophagus is occluded by pinching, the abomasum will also be inflated. Equipment suitable for resuscitation of foals, which should be available wherever there are parturient mares is listed in Table 6.2.

If spontaneous respiration does not commence, then it is necessary to provide positive pressure ventilation. This should be done with the calf, lamb, or foal in sternal recumbency; the person involved blows into the tube and compresses a rebreathing bag attached to an oxygen source. There are now currently available a number of resuscitators for most domestic species. An example is the McCulloch Resuscitator range, an example of one for use in newborn calves, shown in Fig. 6.17A and B; this also has the advantage that, by reversing the pump, fluid can be aspirated from the upper respiratory tract before attempts are made to inflate the lungs using the pump. Overenthusiastic compression of the chest can sometimes cause injury to the ribs and the thoracic organs. In most cases, if resuscitation does not result in spontaneous respiration in 2 or 3 minutes it is unlikely that the newborn will survive, even though there is a good strong pulse and heartbeat. Respiratory stimulants such as coramine and adrenaline are not particularly useful; however, a mixture of solutions of crotethamide and corpropamide placed on the tongue can stimulate respiratory activity in some cases.

Acidosis

The fetus at the time of a normal birth will usually have a mild metabolic and respiratory acidosis; in the case of the former, this is corrected within a few hours, whereas the latter may last up to 48 hours (Szcenci 1985). Dystocia is likely to cause a severe respiratory and metabolic acidosis. Severe acidosis will have an adverse effect on both respiratory and cardiac function, and in the case of the calf will reduce vigour and the suck reflex, resulting in reduced colostrum intake and impaired passive immunity (Grove-White 2000). One of the simplest methods of assessing the degree of acidosis is to determine the time to the calf assuming sternal recumbency. After a normal calving, this was 4.0 +/– 2.2 minutes, whereas after traction, it was 9.0 +/– 3.3 minutes; a time greater than15 minutes was found to have a high predictive value for death of the calf (Schuijt & Taverne 1994). The presence of good muscle tone and a pedal reflex are indicators of a well-oxygenated calf with fairly normal acid–base status. The presence of scleral and conjunctival haemorrhages is indicative of hypoxia and acidosis and carries a poor prognosis; similar lesions are present extensively at necropsy in calves that die at birth (Grove-White 2000).

A calf requiring resuscitation is likely to be suffering from both a metabolic (low plasma bicarbonate concentration) and a respiratory (high PCO_2) acidosis. The PCO_2 will be reduced with improved alveolar gas exchange and tissue perfusion; however, the metabolic acidosis may be treated with sodium bicarbonate (Grove-White 2000). The origin of the metabolic acidosis is due primarily to the production of lactic acid by tissues. When sodium bicarbonate is used to neutralise the acid, CO_2 and H_2O are produced; the former will exacerbate any respiratory acidosis. Thus it is important that the calf is breathing normally so that it can exhale this additional CO_2.

Preferably, treatment of a metabolic acidosis should be carried out after its degree has been assessed after blood gas analysis or the use of the Harleco apparatus. However, under field conditions it is seldom possible, and thus has to be computed according to the recommendations of Grove-White (2000). Thus for a newborn calf whose history and clinical signs suggest that it is acidotic, sodium bicarbonate at a dose rate of 1 to 2 mmol/kg as a bolus intravenous injection of 50 to 100 ml (35 gm in 400 ml of lukewarm water) can be used quite safely.

Injuries at Parturition

Manipulative obstetrical procedures, particularly traction (see Chapters 11 and 14), can result in injury to the newborn. In a study involving the postmortem examination of 327 calves that died during the perinatal period (within 48 hours of birth), 13.2% had fractured ribs, 4.3% diaphragmatic tears, and 2.8% fracture of the spine in the thoracolumbar region (Mee 1993).

Thermoregulation

In the period immediately after birth, the newborn has to adjust to an environment whose temperature may fluctuate widely and which is also usually lower than that of the uterus.

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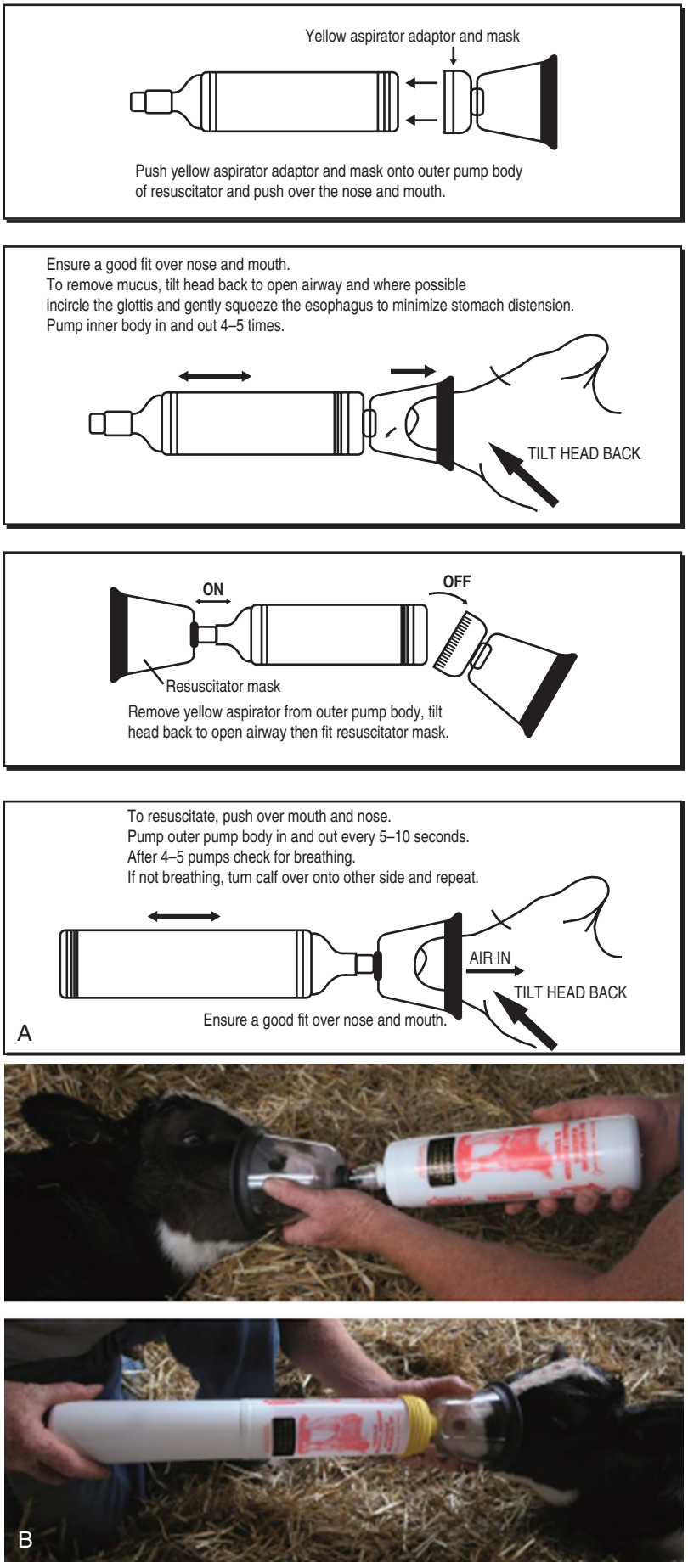

• **Fig. 6.17** (A and B) McCulloch Resuscitator for newborn calf, comprising face mask and manually operated pump. (Courtesy James McIntyre, Dairymac Ltd.)

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After birth the body temperature of the newborn falls quickly from that of the dam before it eventually recovers; the degree of decline and speed of recovery vary from species to species and with the environmental temperature. In the foal and calf, the fall is transient; in the lamb, recovery occurs within a few hours; the piglet takes up to 24 hours or even longer in cold conditions; in the kitten and puppy the period before the temperature recovers to approximately that of birth is 7 to 9 days.

In the newborn, thermoregulation is controlled in two ways. First, the metabolic rate is increased to three times the fetal rate soon after birth. The increased rate is dependent on adequate substrate, and because glycogen and adipose tissue reserves are low in the newborn, it is very important that immediate and adequate food is available. However, the metabolic rate can increase only to a certain level, known as summit metabolism; if this is insufficient to maintain body temperature, then hypothermia occurs (Alexander 1970). The second method of thermoregulation is to reduce heat loss. The newborn has little subcutaneous fat, and hence insulation is poor. The body surface is wet, and thus heat is lost due to evaporation, whereas in species such as the pig, the coat provides little protection. Heat loss is greatest in smaller individuals because they have a greater surface area per unit of body weight.

Thermoregulation in the newborn can be improved in a number of ways:

- Ensure that there is adequate food intake.
- Arrange for birth to occur in at least a thermally neutral environment, and in those species in which thermoregulation is delayed, this environment should be maintained. The newborn puppy should be placed in an environmental temperature of 30° to 33°C for the first 24 hours, which can be reduced to 26° to 30°C by 3 days. Puppies born at a normal room temperature of 18° to 22°C can suffer a fall in rectal temperature of 5°C.
- Reduce heat loss by ensuring that the coat is adequately and quickly dried. A proper nest area should be provided with good insulation and supplementary heating in polytocous species, which will also encourage the huddling together of the litter, thus reducing the overall surface area. In the case of lambs, simple plastic jackets can be an effective way of reducing heat loss.

Umbilicus

At birth the umbilicus usually ruptures passively or in some species, such as the dog, the dam bites through the structure; there are few indications for ligation. Premature severance, especially in the foal, should be prevented because it has been shown that, in the foal, the pulse can persist for up to 9 minutes after expulsion, thereby ensuring an adequate blood volume (Rossdale 1967).

Provided that birth occurs in a clean environment with adequate hygiene, it should not be necessary to handle the umbilicus. However, if there is an outbreak of 'navel ill', it may be necessary to introduce some prophylactic measures. The navel should be carefully cleansed with an antiseptic solution; chlorhexidine is the agent of choice for most species, and then dried.

Nutritional Deficiencies and Infectious Agents

In a study involving the perinatal death of 22 calves born after an unassisted calving, there was some evidence that deficiencies of selenium, iodine, and other trace elements were involved (Mee 1991). When there is a high neonatal mortality rate in the absence of dystocia, the possibility of such deficiencies should be investigated, as well as the possibility of the presence of infectious agents because these may cause not only pregnancy failure but also stillbirth and weakly offspring (see Chapters 24 and 26).

Protection From an Excitable or Vicious Dam

Occasionally, the dam will attack or savage the newborn, in which case it may be necessary to provide some physical protection and resort to the use of tranquilliser drug.

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7

Physiology of the Puerperium

DAVID E. NOAKES

The puerperium is the period after the completion of parturition, theoretically after completion of the third stage of labour (although if the latter is prolonged some of the changes may start before the fetal membranes are voided), when the genital system is returning to its normal nonpregnant state. In the nonseasonally breeding polyoestrous species (the cow, mare, and sow) it is important that the puerperium should be normal because it is the practice under many systems of husbandry to breed from individuals of these species fairly soon after they have given birth. Thus any extension of the puerperium may have a detrimental effect on the reproductive performance of the individual animal concerned. The genital system does not completely return to the original pregravid state because, particularly after the first gestation, certain changes are not completely reversible. Most notable is the size of the cervix and uterus, neither of which ever return to their prepregnant, nulliparous dimensions.

There are four main areas of activity:

- The tubular genital tract, especially the uterus, is shrinking and atrophying as a result of tissue loss; thus reversing the hypertrophy that occurs in response to the stimulus of pregnancy. Myometrial contractions, which continue for several days after parturition, aid this process and help in the voiding of fluids and tissue debris; this process is normally referred to as involution.
- The structure of the endometrium and deeper layers of the uterine wall are restored to their normal nonpregnant state.
- There is a resumption of normal ovarian function in polyoestrous species and a return to cyclical activity.
- Bacterial contamination of the uterine lumen, which is common even after a normal parturition, is eliminated.

Although postpartum infection of the uterus is not uncommon, in some species an acute postpartum inflammatory reaction has been identified in apparently normal healthy animals (see review by Bradford et al. 2015). During the puerperium there are major physiological changes taking place involving the genital system and the reversal of changes associated with pregnancy, as well as changes in the mammary glands in preparation for lactation.

Cattle

Although the stimulus for the changes that occur during the puerperium is primarily due to the removal of the fetus, hormones such as oxytocin and prostaglandin (PG)$F_{2\alpha}$ and inflammatory mediators such as cytokines are also involved. In the case of the latter, however, there is an increase after the end of parturition, in which peak values occur 3 days postpartum and do not return to basal levels until 15 days postpartum (Edquist et al. 1978, 1980).

The puerperium has been studied in detail by Rasbech (1950), Gier and Marion (1968) and Morrow and colleagues (1969), using sequential slaughter and both macroscopic and microscopic examination of the tissues. In recent years, with the advent of new diagnostic imaging techniques such as various ultrasonographic modes, it has been possible to sequentially monitor the changes in tubular genital tract as well as its vascular supply. In addition, it has also been possible to monitor inflammatory markers in the peripheral circulation.

Involution

The reduction in the size of the genital tract is called involution; it occurs in a decreasing logarithmic scale, the greatest change occurring during the first few days after calving. Uterine contractions continue for several days, although decreasing in regularity, frequency, amplitude and duration. The atrophy of the myofibrils is shown by their reduction in size from 750 to 400 μm on the first day to less than 200 μm over the next few days.

Gier and Marion (1968) found that the diameter of the previously gravid horn was halved by 5 days and its length halved by 15 days postpartum. The results of their study are summarised in Fig. 7.1 and show that, after the initial rapid phase of involution, the subsequent changes proceed more slowly. Similar results have been obtained using sequential transrectal ultrasonography (Fig. 7.2). Morrow and associates (1969) recorded a reduction in the rate of involution between 4 and 9 days postpartum, with a period of accelerated change from days 10 to 14 and a gradual decrease thereafter; however, this may have been an artefact. Associated with this phase of rapid involution is uterine discharge. The whole of the uterus is usually palpable per rectum by 8 and 10 days postpartum in primiparae and pluriparae, respectively. The speed of involution of the nongravid horn is more variable than that of the previously gravid horn, which depends upon its degree of involvement in placentation.

There is some dispute about when uterine involution is complete; the differences are probably only subjective. In six studies reported in dairy cattle the time taken for complete involution ranged from 26 to 52 days, whereas in three studies in beef cattle it was 37.7 to 56 days. The changes after 20 to 25 days are generally almost imperceptible.

The cervix constricts rapidly postpartum; within 10 to 12 hours of a normal calving it becomes almost impossible to insert a hand through it into the uterus and by 96 hours it will admit just two fingers. The cervix also undergoes atrophy and shrinkage due to the elimination of fluid and the reduction in collagen and smooth

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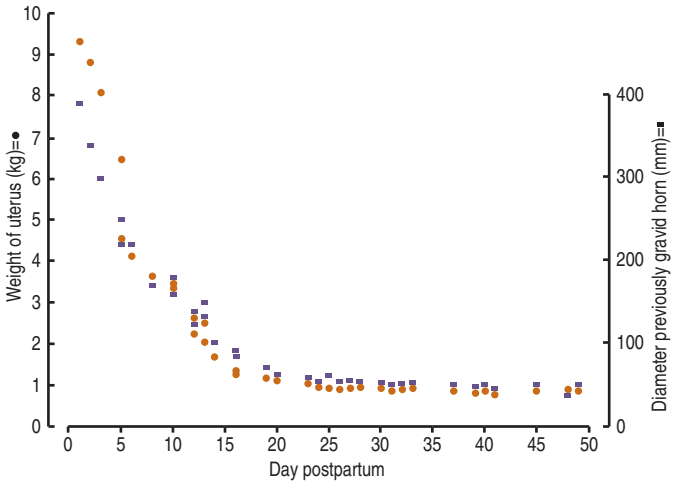
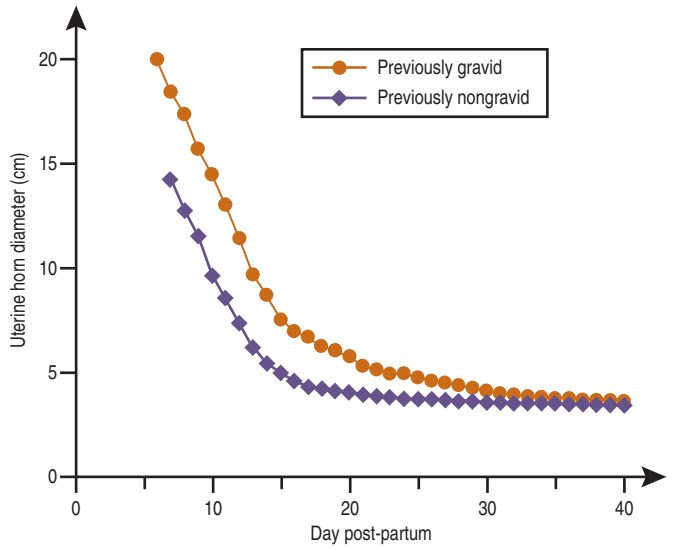


• **Fig. 7.1** Gross changes in the uterus of the cow during the puerperium. (Data from Gier & Marion 1968).



• **Fig. 7.2** Changes in the diameters of the previously gravid and nongravid horns of postpartum cows as determined by B-mode real-time transrectal ultrasonography. (Courtesy Professor I. M. Sheldon.)

muscle. Gier and Marion (1968) found that the mean external diameter was 15 cm at 2 days postpartum, 9 to 11 cm at 10 days, 7 to 8 cm at 30 days, and 5 to 6 cm at 60 days. A useful guide that involution is occurring normally is to compare the diameter of the previously gravid horn with that of the cervix because at about 25 days postpartum the latter starts to exceed the former.

Prostaglandins may have a role in controlling uterine involution, although the postpartum rise in the metabolite of $PGF_{2\alpha}$ (PGFM) may be a reflection of the process of involution rather than the cause. Eley and colleagues (1981) have shown a positive correlation between PGFM concentrations in the peripheral circulation and the diameter of the uterine horn. Using exogenous $PGF_{2\alpha}$ twice daily for 10 days starting from 3 days postpartum, uterine involution has been accelerated by 6 to 13 days; however, the number of animals was small and the frequency and duration of the treatment regimen were very atypical of the normal situation (Kindahl et al. 1982). Because the increase in the uterine mass during pregnancy is due to a combination of increases in both collagen and smooth muscle, then involution must be associated with a reduction of these tissues. This has been shown very clearly in the study of Kaidi and associates (1991) in relation to collagen degradation, but in relation to the loss of smooth muscle the results were equivocal (Tian & Noakes 1991a). The studies of the latter authors also showed that exogenous hormones such as oestrogens, $PGF_{2\alpha}$, and long-acting oxytocin analogues do not influence the rate of involution (Tian & Noakes 1991b). Various dietary supplementations such as beta carotene (Kaewlamun et al. 2011) have been tried in an attempt to hasten involution, but with limited success.

Restoration of the Endometrium

Although placentation in the cow is considered to be of a non-deciduous type, it is well recognised that, during the first 7 to 10 days after calving, there is usually a noticeable loss of fluid and tissue debris. This is sometimes referred to by the herdsperson as the 'second cleansing' or 'secundus'. In human gynaecology the postpartum vulvar discharge is referred to as lochia. The presence of such a discharge in cows is normal, although sometimes individuals will mistake it for an abnormal discharge due to uterine infection and request treatment.

The lochial discharge is usually yellowish brown or reddish brown in colour; the volume voided varies greatly from individual to individual. Pluriparae can void up to a total of 2000 ml, although it is more usually about 1000 ml. In primiparae it is rarely more than 500 ml, and in some animals it is occasionally nil because of the complete absorption of the lochia. The greatest flow of lochia occurs during the first 2 to 3 days; by 8 days it is reduced, and by 14 to 18 days postpartum it has virtually disappeared. At about 9 days it is frequently bloodstained, whereas before it ceases it becomes lighter in colour and almost 'lymph-like'. Normal lochial discharge does not have an unpleasant odour.

The lochia are derived from the remains of fetal fluids, blood from the ruptured umbilical vessels and shreds of fetal membranes, but mainly from the sloughed surfaces of the uterine caruncles. The slough occurs following degenerative changes and necrosis of the superficial layers, first described by Rasbech (1950). The changes that occur are illustrated diagrammatically in Fig. 7.3.

After the shedding of the allantochorion, the caruncle is about 70 mm long, 35 mm wide and 25 mm thick. The endometrial crypts frequently contain remnants of the chorionic villi, which were detached from the rest of the allantochorion at the time of placental separation. Within the first 48 hours postpartum, there is evidence of early necrotic changes in the septal mass of the caruncle; the caruncular blood vessels become rapidly constricted and are nearly occluded. At 5 days the necrosis has proceeded rapidly, so that the stratum compactum is now covered by a leukocyte-laden, necrotic layer. Some of this necrotic material starts to slough and contributes to the lochia. Small blood vessels, mainly arterioles, then protrude from the surface of the caruncle, from which there is oozing of blood, causing a red coloration of the lochia. By 10 days, most of the necrotic caruncular tissue has sloughed and undergone some degree of liquefaction and by 15 days postpartum sloughing is complete, leaving only stubs of blood vessels protruding from the exposed stratum compactum. This eventually becomes smooth by 19 days, as a result of the disappearance of the vessels. A systemic response is observed, probably due to the tissue damage and inflammation associated with the degenerative changes described above, as shown by a rise in the peripheral circulation of acute phase proteins produced by the hepatocytes. These increase rapidly after calving, reaching a peak at 1 to 3 days before declining to basal levels by 2 to 4 weeks (Alsemgeest et al. 1993, Sheldon et al.

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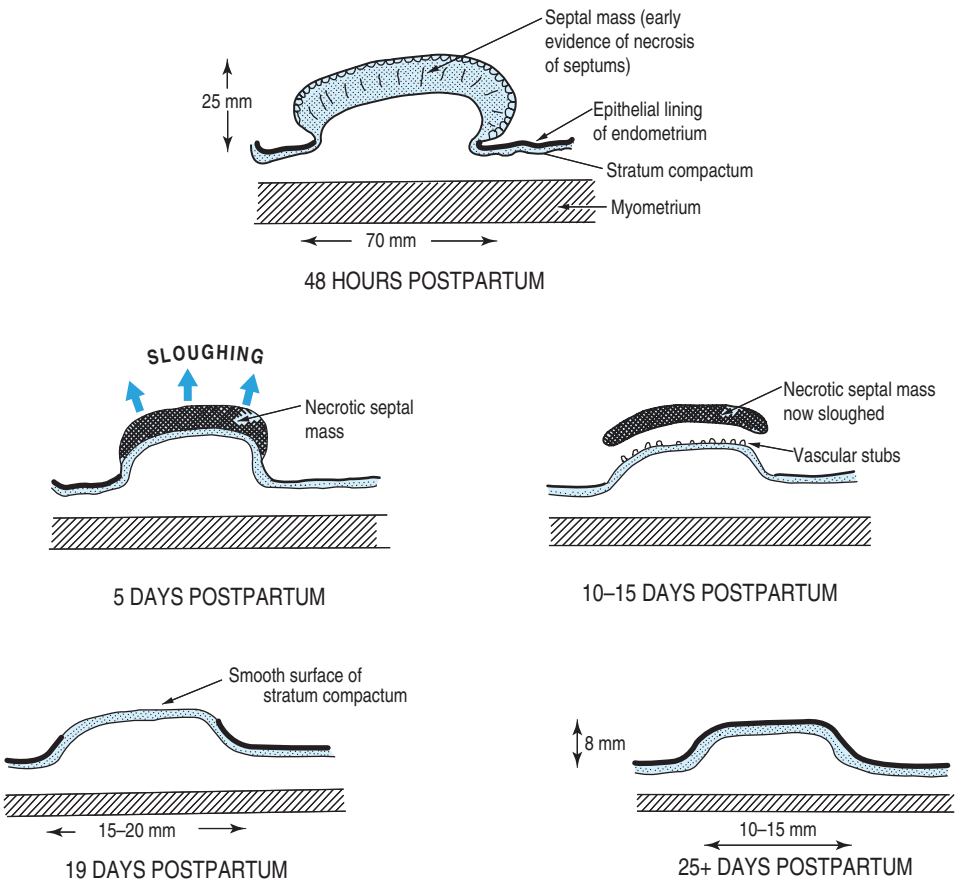

• **Fig. 7.3** The changes that occur in the caruncles of the cow during the puerperium. (Data from Gier & Marion 1968).

2001). Acute phase proteins limit tissue damage and also promote tissue repair (Baumann & Gauldie 1994).

Regeneration of the epithelium of the endometrium occurs immediately after parturition in those areas that were not seriously damaged and is complete in the intercaruncular areas by 8 days. Complete reepithelialisation of the caruncle, which is largely derived from centripetal growth of cells from the surrounding uterine glands, is complete from 25 days onwards, although the stage at which complete healing occurs is variable.

As these changes are taking place, the caruncles are becoming smaller (see Fig. 7.3), so that at 40 to 60 days they consist of small protrusions, 4 to 8 mm in diameter and 4 to 6 mm high. Caruncles of pluriparae differ from those of nulliparae in that they are larger and have melanin pigmentation and a more vascular base.

Return of Cyclical Activity (Ovarian Rebound)

Except during the last month, anovulatory follicular waves occur periodically during pregnancy, with the emergence of follicles up to a maximum of 6 mm in diameter. However, because of the prolonged period of inhibition during pregnancy, due to the continuous negative feedback effect of high steroid concentrations in late pregnancy (particularly progesterone secreted by the corpus luteum and placenta), the pituitary is refractory postpartum, as demonstrated by a lack of response, immediately postcalving, to the administration of gonadotrophin-releasing hormone (GnRH) (Lamming et al. 1979). Between days 7 and 14 postpartum there is an increase in follicle hormone stimulating (FSH) concentrations (over a 3- to 5-day period), associated with the emergence of the first postpartum follicular wave. Using transrectal ultrasonography from 6 to 8 days postpartum, the first follicular wave can be detected with the first dominant follicle larger than 9 mm in diameter being identified at around 10 days (Savio et al. 1990); this tends to occur earlier in dairy than in beef cows. The consequence of this is that the oestradiol and inhibin produced by the follicles suppresses FSH secretion; thus the growing follicles are faced with a decline in FSH. One follicle, usually the largest of the cohort, develops increased numbers of luteinising hormone (LH) receptors and insulin-like growth factor (IGF)-1 binding protein proteases, which allow the maintenance of high levels of bioactive IGF-1 by degrading IGF-binding proteins (Roche 2006). This remaining large follicle continues to grow because of the local paracrine changes within the follicle, despite the reduction in systemic FSH secretion, which prevents other follicles within the cohort from developing. The fate of this follicle (the dominant follicle) is now dependent on the LH-pulse frequency because it is mainly LH-responsive (Fig. 7.4). Thus, according to Roche (2006), one of the following will occur: it ovulates in 30% to 80% of cows; it becomes atretic in 15% to 60% of cows; it becomes cystic in 1% to 5% of cows (see Chapter 24).

Whether or not ovulation occurs is dependent on the following: the size of the dominant follicle; the LH pulse frequency; the concentration of IGF-1. Follicles less than 1 cm rarely ovulate, and the LH pulse frequency required is about one per hour. Thus the first ovulation occurs on average at 21 days in dairy cattle and 31 days in beef cattle (Adams 1999). IGF-1 stimulates follicular

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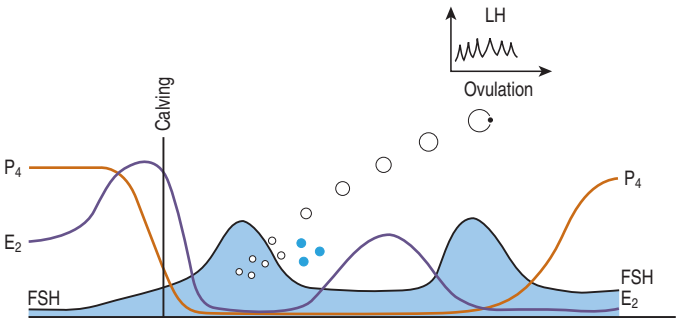

• **Fig. 7.4** The changes in FSH, LH (see inset), oestradiol (E_2) and progesterone (P_4) in relation to the first follicular wave postpartum. (After Roche 2006.)

granulosa cell aromatase activity and thus oestradiol synthesis, therefore potentiating the positive feedback effect of oestradiol on the preovulatory LH surge. After ovulation, there is a luteal phase, which may be of normal length with a return to oestrus after 18 to 24 days, or it may be much shorter, less than 14 days; the latter occurred in 25% of dairy and in 78% of beef cattle (Adams 1999). These short luteal phases probably arise because of premature release of $PGF_{2\alpha}$ arising from the increased oestradiol produced from the formation of the postovulatory dominant follicle on days 5 to 8 of the cycle (Roche 2006). These short luteal phases are more prevalent the earlier the return of normal ovarian activity, i.e., 100% at 0 to 5 days, 60% at 10 to 15 days and 10% at 25 to 30 days postpartum (Terqui et al. 1982).

Many of the ovulations of the first dominant follicle are not associated with behavioural signs of oestrus (so called 'silent heats' or suboestrus; see Chapter 22) (Moller 1970, King et al. 1976, Kyle et al. 1992). This is because the central nervous system requires prior exposure to progesterone to elicit behavioural signs; a similar phenomenon occurs in ewes at the beginning of the breeding season (see Chapter 24). Using continuous time-lapse video recording of herds, 50%, 94% and 100% of cows were identified in oestrus at the first, second and third postpartum ovulations (King et al. 1976); however, with daily observations the frequencies of detected oestrus were only 16%, 43% and 57%, respectively. Sequential milk progesterone assays have enabled the onset of cyclical activity to be determined by the presence of elevated progesterone concentrations. In a survey of 533 dairy cows in four herds (Bulman & Wood 1980), nearly half (47.8%) of the cows had resumed normal cyclical ovarian activity within 20 days of calving, and by 40 days this had increased to 92.4%. In this study only 4.9% appeared to have a delayed return to cyclical activity, i.e., had not returned by 50 days postpartum, and 5.1% of the cows subsequently ceased normal cyclical activity having initially returned to oestrus. A small number, 1.9%, had prolonged luteal activity, presumably due to a persistent corpus luteum or luteal cyst (see Chapter 24). These ovarian abnormalities depressed fertility, as measured by the calving to conception interval, which was 98 days for those with a delayed start to ovarian activity, 102 days for those with persistent luteal function and 124 days for those cows in which there was cessation of cyclical activity, compared with 85 days for normal cows (see Chapter 24). Cows with either shorter or longer luteal phases than normal, resulting in shorter or longer cycles and atypical progesterone patterns before the first service, have longer calving to conception intervals, more services per conception and lower first-service pregnancy rates (Lamming & Darwash 1998). The incidence of prolonged cycles has increased from 3% in the mid-1980s to 11% to 22% in the late 1990s and early 2000s (Roche 2006).

The uterus also exerts an influence on ovarian function because it has been known for some time that the majority of ovulations postpartum occur in the ovary contralateral to the previously gravid horn (Gier & Marion 1968) – the effect being less the later ovulation occurs. It has also been shown that PGFM usually returns to normal levels before the first postpartum ovulation (Thatcher 1986). Similarly, the ovario–uterine axis exerts an inhibitory effect on pituitary LH secretion during the early postpartum period; experimental hysterectomy results in a rapid increase in plasma gonadotrophin concentrations (Schallenberger et al. 1982). There is substantial evidence of an interaction between the uterus and ovaries postpartum, as shown by the observation that the ovary on the side adjacent to the previously gravid horn (the ipsilateral ovary) is less active compared with the contralateral ovary (Nation et al. 1999). The majority of first dominant follicles (70% to 82%) and thus ovulations occur in the ovary contralateral to the previously gravid horn (Nation et al. 1999), with the effect persisting for 20 to 30 days postpartum. The consequence of the difference in folliculogenesis between the two ovaries is that larger follicles on the ipsilateral ovary are associated with better fertility, which is associated with shorter calving–conception intervals and improved pregnancy rates (see Chapter 24; Bridges et al. 2000, Sheldon et al. 2000). As will be discussed later in this chapter, the postpartum uterus in most cows (more than 90%) is contaminated with a wide range of bacteria; there is good evidence that the bacterial load has an influence on folliculogenesis. Sheldon and associates (2002) found that when bacterial growth scores were high on day 7 and 21 postpartum, fewer first or second dominant follicles were selected in the ipsilateral than in the contralateral ovary, respectively. Furthermore, the diameter of the first dominant follicle was smaller, slower growing and had reduced oestradiol secretion in cows with a high bacterial score on day 7 postpartum. Because no effect was seen on FSH secretion or follicular wave emergence, it is likely that this effect is mediated locally. However, bacterial endotoxins and intermediary cytokines have been shown to exert an influence at both the hypothalamic and anterior pituitary level (Peters & Lamming 1990; Williams et al. 2001).

Adrenocorticotrophic hormone (ACTH) (Liptrap & McNally 1976) and corticosteroid administration (da Rosa & Wagner 1981) suppress the secretion of LH. Stimulation of the teat and removal of milk cause a rise in glucocorticoids (Wagner & Oxenreider 1972, Schams 1976). Suckling, which is known to delay the return of cyclical ovarian activity, may exert its effect by modifying the tonic release of GnRH and LH by the release of opioid peptides. The role of prolactin is equivocal for, although bromocriptine treatment during lactation had little or no effect on LH release in cows, there appears to be a reciprocal relationship between the hypothalamic control of LH release and prolactin release. Opioid antagonists increase LH and decrease prolactin secretion; the effects of the agonists are the reverse. The mammary gland has also been shown to have an endocrine role (Peters & Lamming 1990).

Elimination of Bacterial Contamination

At calving and immediately postpartum, the vulva is relaxed and the cervix is dilated, thus allowing bacteria to gain entry into the vagina and thereafter the uterus. A wide range of bacteria may be isolated from the uterine lumen; Elliott and associates (1968) identified 33 different species, those most frequently isolated being *Trueperella* (formerly *Arcanobacterium*) *pyogenes, Escherichia coli*,

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streptococci and staphylococci (Johanns et al. 1967, Elliott et al. 1968, Griffin et al. 1974). Recent studies (Gilbert & Santos 2016) have shown that in the first 7 days postpartum, E. coli were most frequently isolated, but was uncommon after that time, whereas *T.pyogenes* were the most prevalent at 3 weeks and were more likely to be isolated from cows from which E. coli had been previously isolated. The presence of *T pyogenes* at three weeks increased the likelihood of concomitant or subsequent infection with gram negative anaerobes. The isolation of *T pyogenes* at 3 weeks postpartum significantly reduced the chances of pregnancy after 150 days in milk, whereas the presence of α–haemolytic streptococci spp at 7 days improved reproductive performance. In earlier studies there was little reference to the presence of anaerobic bacteria, either because anaerobic culture was not performed or because the methods of culture and isolation were not strict enough. A number of studies (Noakes et al. 1991, Sheldon et al. 2002, Sheldon & Dobson 2004, Foldi et al. 2006) have identified the frequent presence of Gram-negative anaerobes, which play an important role in the pathogenesis of metritis/endometritis because of their synergism with other species of bacteria (Ruder et al. 1981, Olson et al. 1984) (see Chapter 25). Table 7.1 shows the three categories of bacteria isolated from the uterus of postpartum cows and their pathogenicity in relation to uterine disease. The most common isolates in this study were: *E. coli*, streptococci, T *pyogenes, Bacillus licheniformis, Prevotella* (formerly *Bacteroides*) species and *Fusobacterium necrophorum* (Sheldon et al. 2002). Griffin and colleagues (1974) stressed that the flora fluctuates as a result of spontaneous contamination, clearance and recontamination during the first 7 weeks postpartum. In all studies, there is a decrease with time in the percentage of uteri from which bacteria are isolated. This is exemplified in the study of Elliott and associates (1968), in which 93% of uteri examined within 15 days of calving were contaminated, compared with 78% between 16 and 30 days, 50% between 31 and 45 days and only 9% between 46 and 60 days. Other studies (Griffin et al. 1974, Sheldon et al. 2002) have shown a similar very high bacterial contamination rate during the first 20 to 30 days postpartum, with a subsequent decline so that, in normal, healthy cows, the uterus is usually sterile by 6 to 8 weeks postpartum.

TABLE 7.1 Categorization of bacteria isolated by aerobic and anaerobic culture of uterine swabs, based on their potential pathogenicity

BACTERIAL CATEGORY		
1	2	3
Trueperella pyogenes	Acinetobacter spp.	Aerococcus viridans
Prevotella spp.	Bacillus licheniformis	Clostridium butyricum
Escherichia coli	Enterococcus faecalis	Clostridium perfringens
Fusobacterium necrophorum	Haemophilus somnus	Corynebacterium spp.
Fusobacterium nucleatum	Mannheimia haemolytica	Enterobacter aerogenes
	Pasteurella multocida	Klebsiella pneumoniae
	Peptostreptococcus spp.	Providencia rettgeri
	Staphylococcus aureus (coagulase-positive)	Providencia stuartii
	Streptococcus uberis	Proteus spp.
		Propionibacterium granulosa
		Staphylococcus spp. (coagulase-negative)
		α-haemolytic streptococci
		Streptococcus acidominimus
		Coliforms
		Aspergillus spp.
		Fungi
		Bacteroides spp.
		Aeromonas spp.

Categories: (1) recognized uterine pathogens associated with uterine endometrial lesions; (2) potential pathogens frequently isolated from the bovine uterine lumen and cases of endometritis but not commonly associated with uterine lesions; (3) opportunist contaminants transiently isolated from the uterine lumen and not associated with endometritis.
Data from Sheldon et al. 2002.

Blood, cell debris and sloughed caruncular tissue provide an ideal medium for bacterial growth; however, in most cases the bacteria do not colonise the uterus to produce a metritis/endometritis (see Chapter 25). The main mechanism involved in the elimination of the bacteria is phagocytosis by migrating leukocytes, the bacteria being ingested and then killed intracellularly by enzymes, reactive oxygen species, nitric oxide, proteases, and phospholipids released by the cytoplasmic granules (Sheldon 2004). In addition, the phagocytes also release proinflammatory cytokines such as tumour necrosis factor α and interleukins, which stimulate the acute phase protein response. Persistence of uterine contractions (for up to 4 days), sloughing of caruncular tissue, and uterine secretions all assist in the physical expulsion of the bacteria. In addition, the early return to cyclical activity is probably important because the oestrogen-dominated uterus is more resistant to infection. However, there is evidence that in some cases early return to oestrus may be disadvantageous (Olson et al. 1984; and see Chapter 25) in that, if the bacteria are not eliminated at the first oestrus, then the cow enters the first luteal phase in which progesterone is the dominant hormone.

Factors Influencing the Puerperium

Uterine Involution

Many of the methods used to measure the rate of involution have been largely subjective and thus inaccurate; however, with the advent of transrectal ultrasound imaging, accurate measurements of uterine and cervical dimensions are now possible (Okanu & Tomizuka 1987, Tian & Noakes 1991a, Risco et al. 1994, Sheldon et al. 2000, Mateus et al. 2002). Some of the factors include the following:

- **Age.** Most observers have found that involution is more rapid in primiparae than pluriparae.
- **Season of year.** Equivocal, but if there is any influence, involution is probably most rapid in spring and summer.
- **Suckling vs milking.** Results are contradictory; it may be a breed influence on the effect of time to return of cyclical ovarian activity.
- **Climate.** There is evidence that heat stress can accelerate and inhibit the speed of involution.
- **Periparturient abnormalities.** Dystocia, retained placenta, hypocalcaemia, ketosis, twin calves, and metritis delay involution. Periparturient problems cause an overall delay in the completion of this process of 5 to 8 days (Buch et al. 1955, Tennant & Peddicord 1968, Maizon et al. 2004).
- **Subclinical hypocalcaemia**. Delayed reduction in uterine horn length (Heppelmann et al. 2015).
- **Delayed return to cyclical ovarian activity.** Both a cause and an effect because there is good evidence that the uterus can also influence the ovary (see previous mention and Bridges et al.

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2000, Sheldon et al. 2000) as well as the ovary influencing the uterus.
- **Metritis.** This affects uterine diameter.

Restoration of the Endometrium

Retained fetal membranes and metritis inhibit healing, whereas ovarian rebound to cyclical activity may have an influence.

Return of Cyclical Activity (Ovarian Rebound)

Factors that may cause delay include the following:
- **Periparturient abnormalities.** A number of authors have shown that a whole range of periparturient problems delay ovarian rebound.
- **Milk yield.** There is much contradictory evidence on the influence of current milk yield; some authors have demonstrated an effect of the lactation preceding calving. It is frequently difficult to differentiate the influence of nutrition and milk yield.
- **Nutrition.** In both beef suckler and dairy cows, inadequate feeding, especially of energy, during the dry period and after calving inhibits ovarian rebound. This will usually be shown as poor body condition score. Ovulation of the dominant follicle will occur after 3.2 ± 0.2 or 10.6 ± 1.2, follicular waves in beef cows in good or poor body condition score, respectively (Crowe, personal communication, 2000). The effect of nutrition on ovarian function is likely to be mediated by insulin, IGFs and leptin (see Chapters 1 and 24).
- **Breed.** There is a longer delay in beef compared with dairy cows as well as evidence of a breed effect within the two groups, especially in the former.
- **Parity.** Most observers have recorded a delay in primiparae compared with pluriparae – up to the fourth lactation. Conflicting opinions have probably arisen because of the problems of separating the influences of nutritional status, milk yield, and weight loss.
- **Season of the year.** There is good evidence that photoperiod has an effect. This has been shown by experimentally subjecting heifers to continuous darkness, which inhibited the return of cyclical activity (Terqui et al. 1982). Peters and Riley (1982) showed that suckler cows that calved between February and April were acyclic significantly longer than those that calved between August and December. By stimulating the effects of short day length using exogenous melatonin, it has been possible to delay the return to oestrus and ovulation in postpartum beef cows (Sharpe et al. 1986).
- **Climate.** Cows in tropical climates show a delay compared with those in temperate zones.
- **Suckling intensity and milking frequency.** The greater the frequency of milking and the intensity of suckling (number of calves), as well as calf presence, the longer the period of acyclicity. This can be reversed in beef suckler cows by restricting the access of the calf to suckle from 30 days postpartum.

Elimination of Bacterial Contamination

Failure to do this will result in the development of metritis and endometritis (see Chapter 25). Factors that delay this include the following:
- **Magnitude of bacterial contamination:** a massive bacterial flora may overwhelm natural defence mechanisms.
- **Nature of bacterial flora:** Many obligate Gram-negative anaerobes, such as Fusobacterium necrophorum and Prevotella spp., exhibit synergy with Gram-positive aerobic contaminants.
- **Delayed uterine involution**
- **Impaired uterine defence mechanisms**
- **Retained fetal membranes**
- **Calving trauma to the uterus**
- **Return of cyclical ovarian activity:** There is contradictory evidence because, with an early return to oestrus, there is an early oestrogen peak, which should assist in the elimination of the bacteria. However, if the level of contamination is such that a significant bacterial flora persists after the first oestrus, the subsequent luteal phase may allow the bacteria to proliferate (Olson et al. 1984).

Horse

The puerperium is shorter in the mare than in the cow, with rapid involution and relatively good conception rates at the first postpartum oestrus. One of the early papers published by Gygax and associates (1979) is worthy of reading.

In pony mares it is usually possible to identify the outline of the uterine body and horns by rectal palpation at about 12 hours postpartum; in thoroughbreds it is longer. Studies have shown that the uterine horns reach their pregravid size by 30 to 32 days postpartum, and the cervix remains slightly dilated until after the first oestrus. Using transrectal ultrasonography, it has been shown that involution is very rapid between days 1 and 3 postpartum, the reduction being less rapid after day 7. It was complete in the previously gravid horn by day 24 and nongravid horn by day 21, as shown by the absence of any further reduction in size (Lemes et al. 2017). Lochial discharge is relatively slight in most mares and usually ceases by 24 to 48 hours after foaling, although in a few cases it can persist for up to a week. One interesting feature of the mare's uterus postpartum is that fluid accumulates in the uterine lumen. Lemes and colleagues (2017) found that, although no fluid could be detected using transrectal ultrasonography on the first day postpartum, it was present in all of their mares by day 3, thereafter gradually disappearing so that by day 16 it was present in only 1 of the 10 mares examined sequentially. Interestingly, no fluid was observed in any mare by 3 days after ovulation. The cervix remains slightly dilated until after the first oestrus.

Ovarian rebound is rapid, the foal heat occurring 5 to 12 days postpartum. Evidence of follicular activity can be determined as early as the second day. Although conception rates at this first oestrus are lower than at other times, because uterine involution and endometrial repair is not complete, remarkably a large number of mares are fertile, which proves that the endometrium is capable of sustaining a pregnancy. Andrews and McKenzie (1941) found that the endometrium was fully restored by 13 to 25 days postpartum.

There is nothing comparable with the degeneration and sloughing of the endometrium that occurs in ruminants. On the day of foaling, the microcaruncles (see Chapter 4) of the endometrium are unchanged; the stratum spongiosum compactum is oedematous, with a small number of distended glands. However, by day 1 postpartum, there are obvious signs of degenerative changes in the microcaruncles and endometrial glands, and by 2 to 5 days the epithelium of the microcaruncles shows evidence of cytoplasmic vacuolisation, karyorrhexis and an inflammatory reaction with neutrophils and phagocytic cells. On day 7 postpartum the endometrium is similar to that of a mare just before oestrus, with the whole regenerative process complete by 9 to 10 days, when the histological structure is typical of that of a mare in oestrus (Gomez-Cuetara et al. 1995). The maternal crypts disappear as a result of lysis and shrinkage of the epithelial cells of the endometrium, with

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condensation of their contents and collapse of the lumen of the crypt. Gygax and associates (1979) reported that the endometrium is usually quite normal, apart from some pleomorphism of the luminal epithelium at 14 days, but in some mares inflammatory changes may persist for several weeks. Compared to ruminants, the uterus returns to its normal nonpregnant state very quickly, thus being able to sustain and support pregnancy following early mating postpartum.

As in the cow, bacterial contamination of the uterus from the environment is a frequent occurrence. In study by Purswell and colleagues (1989), in 7 out of 13 mares swabbed (54%) aerobic bacteria were isolated from uterine swabs at foaling; the species most frequently isolated being streptococci spp. *Truperella pyogenes* and coliforms. Gram-negative anaerobes were isolated from 3 of 13 (23%). By the first foal heat, in 3 out of 13 mares aerobes were isolated, and by the second foal heat all swabs were sterile. Thus most bacteria are eliminated at the foal heat, and by the second postpartum oestrus in normal healthy mares, all of them are eliminated.

Retained fetal membranes delay involution, although exercise is said to hasten it. The process is more rapid in primiparae than in pluriparae.

Sheep and Goat

The puerperium in both these species is very similar to that in the cow, being typical of ruminants in general. The main difference is that, because they are both seasonal breeders, parturition is followed by a period of anoestrus. There is less information available for the doe, but changes are similar in the two species.

Involution

There is rapid shrinkage and contraction of the uterus, particularly during the third to tenth days postpartum, as determined by measurements of uterine weight and length, diameter of uterine body and previously gravid horn. According to these measurements, involution is complete in the ewe by 20 to 25 days (Uren 1935, Hunter et al. 1968, Foote & Call 1969). In the doe goat, as determined by sequential slaughter, involution is complete by 28 days (Tielgy et al. 1982), although using a combination of slaughter and transabdominal ultrasonography involution was found to be complete by 19 days in Balady goats (Ababneh & Degefa 2005). Using sequential radiography and radio-opaque markers, uterine involution has been shown to be complete by about 28 days in suckling ewes, although an unexplained increase in uterine dimensions has been reported at 42 days (Tian & Noakes 1991b, Regassa & Noakes 1999). Using sequential transrectal and transabdominal B-mode ultrasonography little change in uterine size occurred after day 17, but it was not complete until day 30 (Hauser & Bostedt 2002). Involution is largely due to collagen breakdown because, although tissue collagen concentrations remain fairly constant with advancing pregnancy, there is a seven- to eightfold increase in uterine mass; the reduction in size can only be a reversal of this process.

Restoration of the Endometrium

As in the cow, there are profound changes in the structure of the caruncles with degeneration of the surface, necrosis, sloughing, and subsequent regeneration of the superficial layers of the endometrium. There is evidence, determined by the slaughter of animals 3 days before the expected date of lambing, of prepartum hyaline degenerative changes. This occurs in the connective tissue at the base of and adjacent to the endometrial crypts and also involves both directly and indirectly the walls of the arteries and veins, thus reducing their lumens; the fetal villi are unaffected (Van Wyk et al. 1972).

After dehiscence and separation of the placenta, there is further hyaline degeneration of caruncular tissue, which results in constriction of the blood vessels at the base of the maternal crypts. There is necrosis of the surface layer of the caruncle so that, at about 4 days postpartum, the most superficial layers are undergoing autolysis and liquefaction, which are responsible for the dark reddish brown or black coloration of the lochial discharge at this time. By 16 days postpartum, necrosis of the whole superficial part of the caruncle has occurred with, in most cases, separation of the brown necrotic plaque so that it is lying free in the uterine lumen. The caruncles now have a clean, glistening surface, and the process of regeneration is completed by the reepithelialisation of the caruncles by about 28 days. Similar timing of events has been reported by Gray and associates (2003); however, they also found that the tissue remodeling also occurred in the intercaruncular regions, as well as in the more obvious caruncles. As in the cow, there is also a rise in acute phase proteins in the peripheral circulation, which may well reflect a systemic response of the hepatocytes to the degenerative and inflammatory changes in the caruncles or the presence of bacterial contamination (Regassa & Noakes 1999). They increase rapidly after lambing, before declining 2 to 3 weeks later.

The quantity of lochia voided is variable. Initially, it arises from blood, fetal fluids and placental debris, but as the puerperium proceeds the liquefied, sloughed caruncular tissue is the main source.

Return of Cyclical Activity (Ovarian Rebound)

Although in temperate climates ewes normally become anoestrus after lambing, there are numerous reports of ovarian activity occurring within a few days to 2 weeks postpartum; Gray and associates (2003) found that oestrogen concentrations in the peripheral serum were high at the time of parturition, declined rapidly to day 4 postpartum but then peaked at day 6. Thus follicular growth is common, but ovulation is unusual; when it does occur it is usually associated with a silent heat. Failure of follicular maturation and ovulation is probably due to inadequate release of LH as a result of a deficiency in GnRH synthesis and secretion. As a result, basal LH levels and the pulse frequency of episodic LH secretion are inadequate to stimulate normal ovarian function (Wright et al. 1981). It is possible that the time of the year when the ewes lamb has a profound effect, with those that lamb early and within the normal breeding season being more likely to have normal ovarian rebound. Hafez (1952) has suggested that it is most likely to occur in those breeds that have a longer than average breeding season.

Elimination of Bacterial Contamination

Although it might have been expected that there would be a similar pattern of bacterial contamination to that previously described for the cow and mare, bacteria could not be isolated from uterine swabs obtained from 10 ewes, 1 to 14 days postpartum, at surgical hysterotomy. More recently, using sequential transcervical swabbing of 13 ewes during the first week postpartum, bacteria were isolated from four ewes; thus in the other nine ewes the uterus was sterile (Regassa & Noakes 1999). Similar results were described by Ababneh and Degefa (2006) in Balady goats in their study, with

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bacterial contamination of the uterus, but not the vagina, absent by 10 days postpartum. Until then, E. coli and staph aureus were the most common isolates.

Pig

There are a number of studies that describe the changes that take place during the puerperium of the sow (Palmer et al. 1965, Graves et al. 1967, Svajgr et al. 1974). It is important that the changes should occur rapidly, with a return to a normal pregravid state, so that pregnancy can be established as quickly as possible after weaning.

Involution

Apart from the rapid initial uterine weight loss, which occurs in the first 5 days postpartum, involution is fairly uniform and is complete by 28 days. After day 6, most of the loss of weight is due to changes in the myometrium, notably a reduction in cell numbers, cell size and amounts of connective tissue. Reduction in the latter can be determined by measuring collagen degradation markers in urine, notably hydroxylsyl pyridinoline (HP), as well as lysyl pyridinoline (LP) that occurs mainly in bone. The results were consistent with a postpartum increase of soft tissue collagen catabolism because bone has a low HP:LP ratio of 4 whereas soft tissues such as the uterus have a high HP:LP ratio of 20 or higher because they contain only trace amounts of LP, which is related in parallel with uterine involution (Belstra et al. 2005).

Restoration of the Endometrium

The uterine epithelium 1 day after farrowing is of a low columnar or cuboidal type, and there is evidence of the extensive folding that is present during pregnancy. The epithelial cells at 7 days are very low and flattened and show signs of degenerative changes; however, there are also signs of active cell division, which is subsequently responsible for regeneration of the epithelium. This latter process is complete by 21 days and is capable of sustaining pregnancy.

Return of Cyclical Activity (Ovarian Rebound)

Suckling and subsequent weaning have a profound effect upon ovarian rebound and indirectly on other puerperal changes in the genital tract. In most cases there will be no return to oestrus and ovulation until the piglets are removed. In the study by Palmer and associates (1965), there was no evidence of ovulation during suckling periods of up to 62 days. In general, the later the time of weaning, the shorter the time interval to the first oestrus; for example, if the litter is weaned at 2, 13, 24 and 35 days postpartum the mean times to first oestrus were 10.1, 8.2, 7.1 and 6.8 days respectively (Svajgr et al. 1974). The time to the first ovulation can also be shortened by the temporary removal of the whole litter for varying periods during the day (partial weaning) or the permanent removal of part of the litter (split weaning) (Britt et al. 1985; Chapter 33).

There is rapid regression of the corpora lutea of pregnancy, with signs of cellular degeneration by 3 days postpartum, so that by day 7 they consist mainly of connective tissue. There is considerable follicular activity during suckling, with follicles sometimes reaching a diameter of 6 to 7 mm. This is sometimes associated with behavioural oestrus shortly after farrowing but in no cases is there ovulation: the follicles become atretic.

In a study of the endocrine changes of the postpartum sow, Edwards and Foxcroft (1983) showed that, irrespective of whether weaning occurred at 3 or 5 weeks, the great majority of sows showed a preovulatory LH surge within 7 days of weaning. At the time of weaning there was a transient rise in basal LH of about 2 days' duration but, unlike the cow, there was no consistent change in the episodic release of LH. Prolactin concentrations are high during lactation but decline rapidly to basal levels a few hours after weaning; mean FSH concentrations rise 2 to 3 days after weaning. Follicular growth and ovulation are inhibited during lactation because of suppressed LH secretion; this probably occurs as a result of direct neural inhibition of GnRH synthesis and release. Inadequate nutrition, particularly severe weight loss, can delay the onset of cyclical ovarian activity, as can the season of the year (Britt et al. 1985). It is generally accepted that exposure to the boar has the reverse effect.

The time of weaning, and thus the time of first oestrus, also has other effects on reproductive function, owing to the time taken for the completion of the puerperium. Fertilisation rates and pregnancy rates are improved the later the time of weaning, and hence the later sows are served after farrowing.

Dog

Because the bitch is monocyclic, parturition is followed by anoestrus, the onset of the next heat being unpredictable. Regression of the corpora lutea of pregnancy is initially rapid, so that by 1 or 2 weeks postpartum they have been reduced in size. However, thereafter it is much slower, so that even after 3 months the nonfunctioning corpora lutea measure 2.5 mm in diameter.

The rate of involution is similar to that of other species, and the uterine horns are restored to their pregravid size by 4 weeks. The lochial discharge immediately postpartum is very noticeable because of its green colour due to the presence of uteroverdin; unless there are complications, this should change to a blood-stained, mucoid discharge within 12 hours. In some bitches a blood stained vulvar discharge can persist many months postpartum; this is due to subinvolution of the endometrium where the placenta was attached and was first described in 1966 by Beck and McEntee. In a large study in which reproductive tracts were examined from 95 bitches, 20 had subinvolution of the placental sites. On gross examination the affected sites were haemorrhagic in appearance and about twice the size of the normally involuted sites. Microscopically, large masses of collagen, haemorrhage, and dilated endometrial glands were present. In one study (Voorhorst et al. 2013) a discharge persisted for many months in one bitch – 270 days postpartum. The same authors found that treatment with low doses of megestrol acetate (0.1 mg/kg) per day for one week followed by half the dose for the second week was successful in eliminating the discharge.

In the nonpregnant bitch, the surface of the endometrium undergoes desquamation followed by regeneration, with repair completed by 120 days after the onset of oestrus (see Chapter 1). After pregnancy and normal parturition the time taken for regeneration of the endometrium is about 2 weeks longer. The areas of previous placental attachment are dark green/grey in colour, subsequently becoming dark brown and visible macroscopically up to 12 weeks postpartum. Desquamation of the epithelial lining of the endometrium starts at 6 weeks postpartum and is complete by 7 weeks; the whole process of regeneration has ended by 12 weeks, when involution can be considered to be complete (Orfanou et al. 2009).

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Cat

Studies on uterine involution and restoration of the endometrium during the puerperium show that they are more rapid than in other species. One study involving Turkish Van cats, using transabdominal ultrasonography, suggested that morphological involution was complete as early as 5 to 6 days (Sendag et al. 2016). Queen cats will normally have an obvious serosanguinous vulvar discharge for up to a week or more after kittening, gradually declining so that by about 2 weeks it will have disappeared. Transabdominal ultrasonography has shown that uterine involution is complete by 25 days postpartum when the dimensions become comparable with those of anoestrus (Blanco et al. 2015). Lactation will usually suppress oestrus effectively (Schmidt et al. 1983), but if the queen has no kittens to suckle, or only one or two, she may show a postpartum oestrus 7 to 10 days after parturition, and thus if mated she may conceive.

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8

Pharmacological Agents in the Control of Reproduction

RICHARD LAVEN

Manipulation of normal cyclic activity can be used simply for convenience, e.g., ensuring a bitch is in oestrus when a particular dog is available for mating or avoiding the need for oestrus detection in a group of dairy heifers kept at pasture in a remote rural location. However, it is most commonly used, especially in livestock, to improve productivity. Potential benefits include: producing offspring out of season in species that are seasonal breeders and also in nonseasonal breeders by reducing the interval from birth to the next pregnancy.

Non-Exogenous Hormone Methods

There are also a variety of methods of manipulating cyclic activity, without using reproductive hormones are available. These include altering exposure to light (which can be mimicked using exogenous melatonin), the timing of weaning, and exposing females to a fertile male.

Light

Ensuring that parturition occurs at a time when resources are available and sufficient to feed the neonate is vital for reproductive success, as is ensuring that, when resources are limited, they are used for functions other than reproduction that are critical for animal survival such as immune functions, thermoregulation and cellular maintenance. This is particularly important in temperate regions where there is significant seasonal variation in the availability of resources.

In such situations the ability to detect variations in photoperiod can be very useful in determining where to put resources. This requires that the animal to be able to reliably estimate current day length and identify whether it is increasing or decreasing. Identifying changes in day length is critical, as without this ability, distinguishing between spring and autumn is impossible. So mammals that respond to photoperiod are often divided into those in which reproductive activities are apparently stimulated to activity by a lengthening photoperiod (e.g., horses and cats) and those in which the stimulus appears to be decreasing day length (e.g., sheep and goats). Interestingly, the process does not appear to be principally one of stimulation but is driven by the development of refractoriness to photoperiod. So, for example, in sheep and goats, the onset of the breeding season in autumn occurs when the animal becomes refractory to the suppressive effect of long day length, whereas in winter the breeding season ends when the animal becomes refractory to the stimulatory effect of the short days (Delgadillo et al. 2011, Walton, et al. 2011). These results suggest that, in seasonal breeders, there is an endogenous circannual rhythm involved in regulating seasonal reproductive changes that is synchronised by changes in photoperiod.

In mares artificial alterations to photoperiod are commonly used to advance the onset of the breeding season. The most commonly used programme is a fixed 15-hour daylight programme, but there are alternatives such as a 2-hour pulse of artificial light starting 9 hours after the onset of darkness (Nagy et al. 2000). As these programmes take 60 to 90 days to be effective, in the Northern hemisphere they need to start in early December in order to maximise the chance of the mare being mated at the optimal time. There is significant individual variation in response, i.e., the timing of the onset of cyclical ovarian activity to these changes in photoperiod. To overcome this and improve the synchronisation of the first ovulation, artificial photoperiod programmes can be combined with exposure to exogenous progesterone for 12 to 15 days at the end of the programme (Nagy et al. 2000).

Artificial alteration of the photoperiod has also been used to control cyclicity in sheep and goats. However, these species need decreasing daylight to stimulate activity. The production of short days in midsummer requires special daylight-proof housing, significantly adding to costs (Chemineau et al. 1992). However, reproductive activity can also be stimulated by exposing animals to artificially long days and then returning them to the natural shorter day; for example, exposing ewes in late pregnancy or at parturition to 22 hours of 'daylight' for at least one month followed by a return to natural day length will stimulate reproductive activity as the exposure to the long days results in the ewes no longer being refractory to the stimulatory effect of the short natural day length (Chemineau et al. 1992). This treatment will result in ewes having a fertile oestrus 3 months after lambing without the need for daylight-proof housing. These regimes seem to be more effective in the doe than the ewe, which may be the result of the increased seasonality in the latter species. However, in both goats and sheep, modifying photoperiod is not used on many commercial farms, often because of the anticipated costs but also because of the wide range of response to such programmes, particularly in sheep.

The Male Effect

Introducing a male into a group of females can induce an oestrus even in animals that were in seasonal anoestrus. This 'male effect'

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has been studied extensively in goats and sheep and is produced in response to a range of signals from the male, including odour and behaviour. Exposure to these signals stimulates LH secretion followed by ovulation and the return of cyclical ovarian activity (Vielma et al. 2009). There is some synchronisation of oestrus, but it is far from complete. Another issue is that, although the most obvious photoperiod response is seen in the doe and the ewe, there are also seasonal differences in male fertility. Out-of-season males can fail to produce the signals that stimulate female cyclicity; this can be prevented by using the same lighting regimes for males that are effective in females (Vielma et al. 2009) or by using melatonin.

Weaning and Lactational Anoestrus

In many mammalian species lactation is associated with suppression of cyclical activity. In the sow lactation has been shown to inhibit the release of LH, although as lactation progresses this inhibition decreases. Weaning, which removes both the suckling-induced neuroendocrine reflexes and the nutritional impact of milk production, results in rapid restoration of cyclicity as the inhibition of LH secretion disappears. If litters from a number of sows are weaned at the same time, this will result in some degree of synchrony of oestrus (see Chapter 33). Early weaning of piglets (3–4 weeks) is commonly used in order to optimise reproductive performance on commercial pig farms. However, this early weaning has significant impacts on piglet health and performance, particularly in the postweaning period (Downing 2015), so there needs to be a focus on inducing oestrus in lactating sows. Several strategies that stimulate sows into oestrus and ovulation are available, including group housing, boar exposure and intermittent suckling (Downing 2015); these are likely to become more important in the future.

Hormonal Control of the Oestrous Cycle

Cyclic activity is a complex process that involves many coordinated effects. As such, there are a large number of stages in which cyclic activity is (or can be) influenced by specific hormones, and thus a large number of hormones have been used exogenously in an attempt to influence normal cyclic activity.

The hormones that are commonly used in these attempts can be divided into six categories: (1) melatonin (described earlier in the chapter); (2) oestrogens; (3) progestagens; (4) prostaglandins; (5) compounds that stimulate the release of anterior pituitary hormones; and (6) compounds that replace or supplement anterior pituitary gonadotrophins.

Melatonin

Photoperiod affects fertility by influencing the synthesis of melatonin (see Chapters 1 and 3), a hormone produced by the pineal gland. The light signal is transmitted from the retina to the pineal gland and produces a circadian rhythm in the synthesis and secretion of melatonin, with high concentrations at night and low concentrations during the day. Melatonin concentration influences a range of physiological processes, including the reproductive functions described earlier, but also appetite and food intake and body temperature.

In species which respond to short days and, consequently, respond to increasing concentrations of melatonin (e.g., sheep and goats), exogenous melatonin has proven to be an effective method of advancing the onset of cyclical ovarian activity. Such regimes are, unsurprisingly, ineffective in 'long-day' breeders (which respond to decreasing melatonin concentrations). As yet, no effective method other than exposure to light has been shown to reliably reduce melatonin secretion in long-day breeders.

In susceptible species melatonin can be supplemented orally or, more commonly, by using a subcutaneous implant. Ewes (and does) need to be separated from males for at least 7 days before the insertion of the implant and then remain separated for 30 to 40 days after insertion. Males should be introduced at this point, even though peak mating activity will occur approximately 60 days after implant insertion. In receptive animals the breeding season can be advanced by 2 to3 months with good fertility. However, the optimal timing of insertion relative to the expected onset of the breeding season is significantly affected by breed, particularly in sheep (Mura et al. 2017). These breed effects are probably related to the differences between sheep breeds in their sensitivity to changes in photoperiod. Melatonin implants can be used in bucks and rams to stimulate reproductive behaviour, but doses required are higher than in the equivalent females. Melatonin should not be used in nulliparous females.

Oestrogens

Oestradiol is an essential part of the hormone cascade that leads to ovulation; it is also responsible for the behavioural changes associated with oestrus as it stimulates sexual receptivity and increases attractiveness to the male. It also has effects on follicular dynamics and corpus luteum (CL) formation. Therefore exogenous oestrogens are an obvious choice of a hormone that should influence cyclical ovarian activity. Both synthetic and natural oestrogens have been used in a wide range of species. However, especially when used alone, the main effects in many species of exogenous oestrogens are changes in the uterus/oviduct and in sexual behaviour, without necessarily influencing ovarian activity and ovulation. A further barrier to the use of oestrogens was the decision in 1999 of the EU Scientific Committee on Veterinary Measures relating to Public Health that oestradiol was carcinogenic, exerting both tumour-initiating and tumour-promoting effects. This led to an immediate ban on the use of oestrogens as growth promoters and, 7 years later, a complete ban on oestrogen use in food-producing animals. This EU ban has led to a large number of countries also banning the use of oestrogens in food animals. However, oestrogens are still approved for use in the US and Canada and are commonly used for oestrus synchronisation in many South American countries (as part of a multihormone programme).

Progestagens

Similar to oestrogens, changes in progesterone concentrations are a critical part of the process that leads to ovulation. Progesterone and synthetic progestagens have therefore been used extensively in most domestic species as a method of controlling the oestrous cycle, particularly for synchronising oestrus activity within groups. In general, the principle behind the use of exogenous progestagens is that they mimic the function of the CL; i.e., they produce negative feedback effects on the anterior pituitary, which suppress cyclical activity by inhibiting the release of gonadotrophin. When the supply of exogenous progestagens ceases, the inhibition disappears and cyclical activity resumes.

Uses in the Horse

In horses, although progestagens have been used to synchronise oestrus, the most common use for these products has been to prevent

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the effects of oestrus on training and performance in competition horses. Van Niekerk et al. (1973) showed that a daily injection of 0.3 mg/kg progesterone was effective in preventing oestrus and that, once treatment ceased, a normal fertile oestrus occurred 3 to 7 days later. There are two major issues with such a protocol: first, multiple daily injections are often not tolerated by the horse, especially as injection site reactions are common with progestagens; and second, progesterone has an anabolic effect that, depending on regulations, can prevent its use in competition horses. This led to the development of potent oral progestagens for use in the horse, the most common of which is allyltrenbolone or altrenogest. Unlike progesterone, this product has no anabolic activity. Altrenogest has been used to do the following:

1. Stimulate the onset of cyclical activity in the early breeding season. To be effective, the treated mare should have some follicle activity. Altrenogest is thus commonly used towards the end of an artificial lighting period as the latter increases the proportion of horses with some follicle activity. Treatment for 10 days at the standard dose rate of 0.044 mg/kg body weight is recommended.
2. Suppress oestrus in mares with prolonged oestrus or other aberrant sexual behaviour. Treatment for 10 days at the standard dose rate of 0.044 mg/kg body weight is recommended.
3. Suppress oestrus in mares with normal oestrus activity. Treatment for 15 days at the standard dose rate of 0.044 mg/kg body weight is recommended.
4. Control the timing of oestrus so that effective use can be made of a stallion or artificial insemination (AI). Treatment for 15 days at the standard dose rate of 0.044 mg/kg body weight is recommended. Once treatment ceases, the mare should come into oestrus 2 to 3 days later.

More recently a long-acting injection of altrenogest has been developed as an alternative to oral supplementation. (McConaghy et al. 2016) found that a single injection of 0.3 mg/kg altrenogest given to mares in oestrus suppressed oestrus within 24 hours and that this suppression lasted at least 6 days. They also showed that a 0.3 mg/kg dose was not associated with any significant injection site reaction.

Other synthetic progestagens such as medroxyprogesterone acetate and norgestomet have been tried in horses but are ineffective, probably because, unlike altrenogest, they do not bind adequately to the equine progesterone receptor (McCue 2016).

Progesterone-releasing devices have also been used to control oestrus in horses. Progesterone implants are not effective, probably because they release progesterone too slowly. Intravaginal devices are more effective and can be used to increase the proportion of mares that become pregnant early in the breeding season (Newcombe 2002), although the degree of synchronisation is often much lower than after oral altrenogest.

Uses in Cattle

Progestagens can be used to suppress oestrus in cows as described for the mare, but such use tends to be specialised (e.g., confined research cattle). However, by far the most common use is to synchronise groups of cows and heifers for artificial insemination, without requiring the need for long periods of oestrus detection.

Progesterone was first used by daily injection to synchronise oestrous cycles in groups of cows in 1948 (Christian and Casida 1948). Initial experiments used long courses of injections (typically 16–21 days) that exceeded the expected lifespan of any naturally occurring CLs. This type of programme resulted in fairly good synchronisation of oestrus 4 to 6 days after the cessation of treatment; however, fertility at the first oestrus was often markedly depressed. The development of orally active progestagens (such as melengesterol acetate) revived interest in progesterone-based synchronisation; however, the poor fertility issue remained. The most likely reason for the poor fertility was impaired sperm transport as a result of the atypical hormone balance after withdrawal of the progestogen. The depression in fertility was inversely related to the length of treatment, but, unfortunately, the degree of synchronisation was positively related.

These issues were solved by combining progesterone with additional hormones. The use of combinations meant that short periods of progesterone supplementation could be used (optimising fertility), as the additional hormones prevent the persistence of naturally-occurring luteal tissue (which would otherwise reduce synchronisation).

One widely used programme is the combination of a subcutaneous implant of the synthetic progestogen, norgestomet, together with the injection of oestradiol valerate. Removing the norgestomet implant after 9 days produces good synchronisation (95% within 5 days of removal), as well as satisfactory conception rates after two inseminations at 48 and 60 hours after removal (Wishart et al. 1977). The key disadvantage with this method is the requirement for a second handling to remove the implant. Nevertheless, this protocol is still in use more than 40 years after being developed, at least in countries in which the use of oestradiol in food animals is permitted.

However, by far the most common method of progesterone supplementation has been the use of intravaginal progesterone-releasing devices. The first device to be developed was a stainless steel coil covered with an inert elastomer (PRID) impregnated with 1.55 G progesterone; this has now been replaced by a polyethylene and ethyl vinyl acetate triangle in order to increase retention rates (Fig. 8.1). Most other intravaginal progesterone-releasing devices are based on a t-shape flexible arm design (Fig. 8.2).

All devices are inserted into the vagina using an introducer, from where the progesterone is released and absorbed into the bloodstream, producing progesterone concentrations in the peripheral blood of between 3 to 5 ng/mL (vanWerven et al. 2013). Plasma progesterone concentrations depend on the type of product used (a higher progesterone content in the product leads to higher initial peripheral progesterone concentrations) and the time since insertion because a longer time results in lower plasma progesterone concentrations. Removal of the device after 7 to 10 days results in oestrus 2 to 3 days later, provided that there is no persistent luteal

• **Fig. 8.1** Progesterone-releasing intravaginal device (PRID) for use in cattle.

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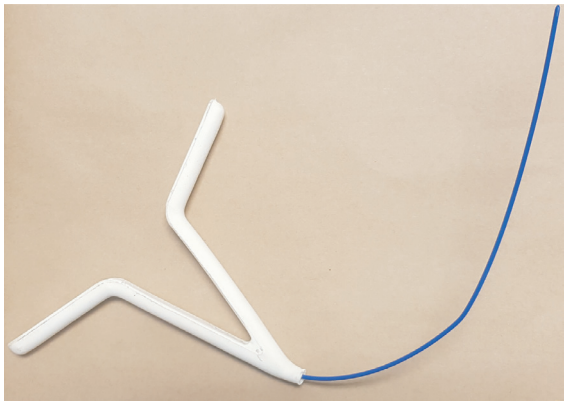
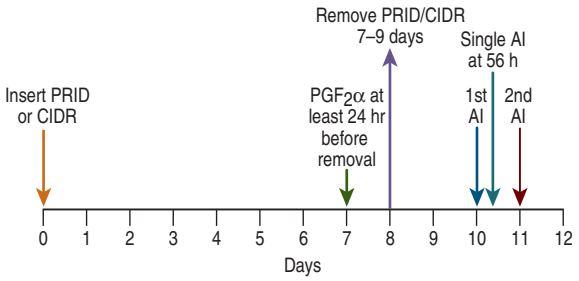


• **Fig. 8.2** Controlled internal drug release (CIDR) device for use in cattle.



• **Fig. 8.3** Protocol used for oestrus-synchronization in the heifer or cow using a combination of either a PRID or CIDR together with Prostaglandin $F_{2\alpha}$ followed by a single or double fixed-time AI (if heat detection not used).

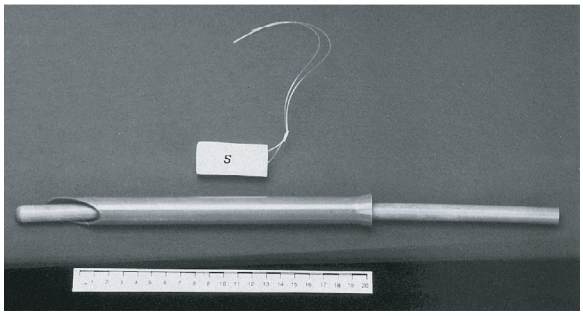
tissue. Initially, oestradiol was injected at the time of insertion to prevent this, but subsequently, $PGF_{2\alpha}$ has been increasingly used, especially after the EU ban on the use of oestradiol. The standard protocol with $PGF_{2\alpha}$ is: insertion of progesterone-device (day 0), followed by $PGF_{2\alpha}$ injection on day 6 and device removal on day 7 (Fig. 8.3). Good conception rates can be achieved following two fixed-time inseminations at 48 and 72 hours after device removal (vanWerven et al. 2013).

Progesterone devices can also be used to synchronise oestrus in nonpregnant cows before being inseminated. For example, in the Fastback programme in the US, a progesterone device is inserted on day 14 after insemination. This is removed on day 21, and on days 22 to 25 the treated cows are observed for oestrus. This increases the proportion of nonpregnant cows that are reinseminated at the first oestrus following the initial insemination. Progesterone-based resynchronisation programmes are also used in New Zealand in cows treated for anovulatory anoestrus. The evidence of efficacy is equivocal and, at the herd level, focusing on reducing the proportion of cows that have anovulatory anoestrus is likely to be more beneficial than resynchronisation (Bates et al. 2016).

Progesterone devices are also useful in treating anoestrous cows. The effectiveness of these devices was first demonstrated by Lamming and Bulman (1976), who showed that 14-days treatment with progesterone alone induced a posttreatment oestrus in 5 out of 7 cows. However, such a programme suffers from the same issue as other long progesterone programmes, i.e., good synchronisation but reduced fertility. Solving this issue requires combination treatments, which are discussed later in this chapter.

Uses in the Sheep

Progestagens have been widely used in controlling reproduction in the ewe, either on their own or in conjunction with other



• **Fig. 8.4** Progestogen-impregnated intravaginal sponge or tampon *(s)* with speculum and introducer. For use in sheep and goats.

• **Fig. 8.5** Controlled internal drug release (CIDR) device for use in sheep and goats.

hormones. They have been used to induce oestrus in the anoestrous ewe during the nonbreeding season and also for synchronisation of groups of ewes that are already showing cyclical activity. The most common route of supplementation is the intravaginal route in the form of impregnated sponges (Fig. 8.4) or t-shaped silicone devices (Fig. 8.5). As in cattle, provided that the progestogen is correctly incorporated into the sponge, it will be readily absorbed at a sufficient rate to ensure a full negative feedback effect on pituitary function. Initially, progesterone was used in the sponges but was superseded by the potent short-acting progesterone-analogues such as fluorogestone acetate (FGA) and medroxyprogesterone acetate (MAP). However, the introduction of small versions of the t-shaped cattle devices such as DICO and CIDR for sheep has meant that progesterone is again being used to control cyclicity in the sheep.

The standard recommendation for intravaginal progestagens in the ewe is a 14-day treatment programme. Outside of the normal breeding season, using eCG at the end of the progesterone–priming period, i.e., on device removal, is essential even if melatonin is used to advance the breeding season. During the breeding season this is not the case, although the addition of eCG will improve synchronisation.

Shorter 6- or 7-day programmes have also been developed. Outside of the breeding season the recommended programme is

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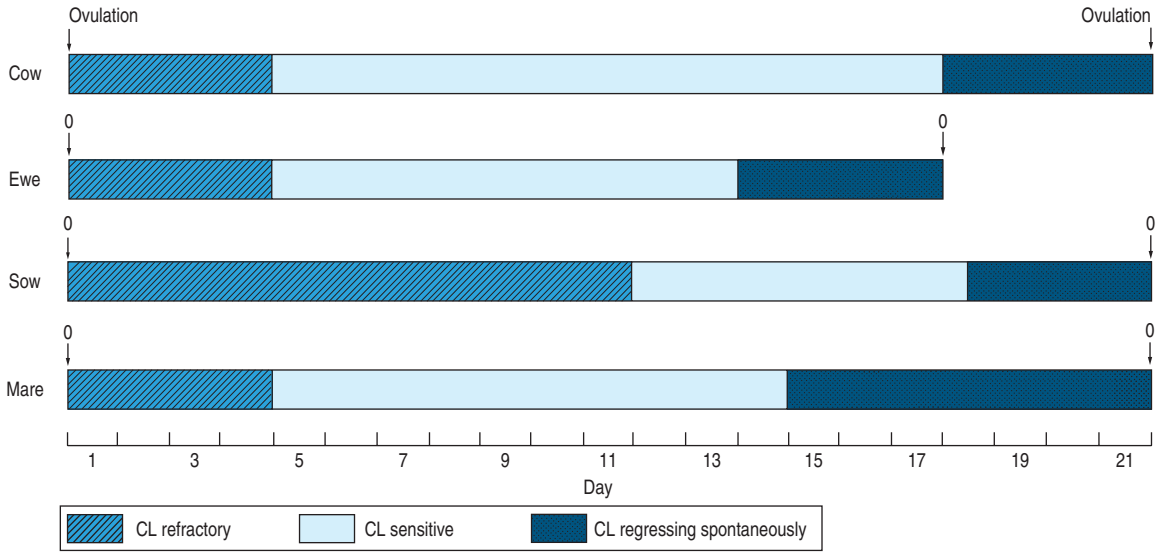

• **Fig. 8.6** Sensitivity of the corpus luteum (CL) of different species to $PGF_{2\alpha}$ during the oestrous cycle.

that the device is removed on day 6, concurrent with the injection of eCG; during the breeding season the addition of $PGF_{2\alpha}$ is required if shorter programmes are to be effective, either at the time of device insertion or 24 hours before removal. Irrespective of the programme, fertility is slightly lower in synchronised sheep that are outside their breeding season than in sheep that are naturally cycling.

Uses in the Goat

The devices used in the goat tend to be the same as those in the sheep. Goats object to the insertion of the sponges more than sheep, so the lower discomfort resulting from the use of t-shaped silicone devices is advantageous. Goats show intense oestrus 36 to 48 hours after device removal. There is no convincing evidence that doses of either progestagens or eCG are different from those recommended in the sheep.

Uses in the Pig

In pigs oral treatment with progestagens has been the only widely used method of progesterone supplementation. Currently, altrenogest is the only progestogen that is available commercially. Details of the use of progestagens in breeding programmes are described in detail in Chapter 33.

Uses in the Dog and Cat

Progestagens have been used for many years to suppress oestrus in both the bitch and queen cat. They act via a negative feedback effect on the hypothalamic–pituitary axis, probably producing their effect by preventing a rise in gonadotrophin secretion, the trigger for the natural progression from stage to stage of the oestrous cycle (England 1998). Progestogen treatment causes a decline in FSH concentrations in the peripheral blood but not LH, although there is a decreased responsiveness to GnRH. Details of the use of progestagens in the dog and cat are described in Chapter 35.

Prostaglandins

The length of the interoestrus interval in most domesticated species is controlled by the life span of the CL; thus removing the CL will induce oestrus. Exogenous $PGF_{2\alpha}$ (or its analogues) can be used to induce luteolysis and manipulate the normal pattern of cyclic activity.

Of the key domestic species the CLs of the cow, mare, sow, ewe, and goat normally respond to the administration of exogenous prostaglandins, but the CLs of the bitch and the queen require repeated doses. Prostaglandins are therefore not of clinical use in the latter two species.

In species which do respond there are differences in when the CLs become responsive to exogenous prostaglandins (Fig. 8.6). In the cow, ewe and goat the new developing CL is generally refractory for 3 to 5 days after ovulation; the mare is similar although large doses of $PGF_{2\alpha}$ may be effective from 2 days after ovulation. Once the natural process of luteolysis has started, exogenous prostaglandins will have no effect; this means that the cow, doe goat, mare, and ewe have a responsive CL for 13, 13, 10 and 9 days, respectively. However, the sow is different because the CL is refractory for up to 11 days after ovulation and is thus responsive for a much shorter period of only 7 or 8 days.

Because prostaglandins are abortifacients, they must not be used in animals that might be pregnant. If there is any doubt, pregnancy diagnosis must be performed.

Uses in Cattle

Prostaglandins have been used successfully to synchronise oestrus in groups of cows and heifers. This technique has applications in beef cows and heifers and also in dairy heifers in which oestrus detection is frequently difficult, thus enabling the routine use of AI at a predetermined time. The availability of artificial insemination in such situations allows the use of semen from genetically superior sires and can thus result in improved genetic potential in the offspring.

Prostaglandin-based programmes can either use oestrus detection to determine the timing of AI or use fixed-time AI. If oestrus detection is feasible, then a group of cows can be injected, and those with a responsive CL will come into oestrus 2 to 7 days later (most coming into oestrus 3–5 days later) and can be inseminated. Those cows which do not respond or which have an unobserved oestrus can be injected again 11 to 14 days later, when all remaining cows, if they are cycling normally, should have a responsive CL (Fig. 8.7). Most cows will show oestrus between 3 to 5 days later and can be inseminated. If oestrus detection is not feasible, then a

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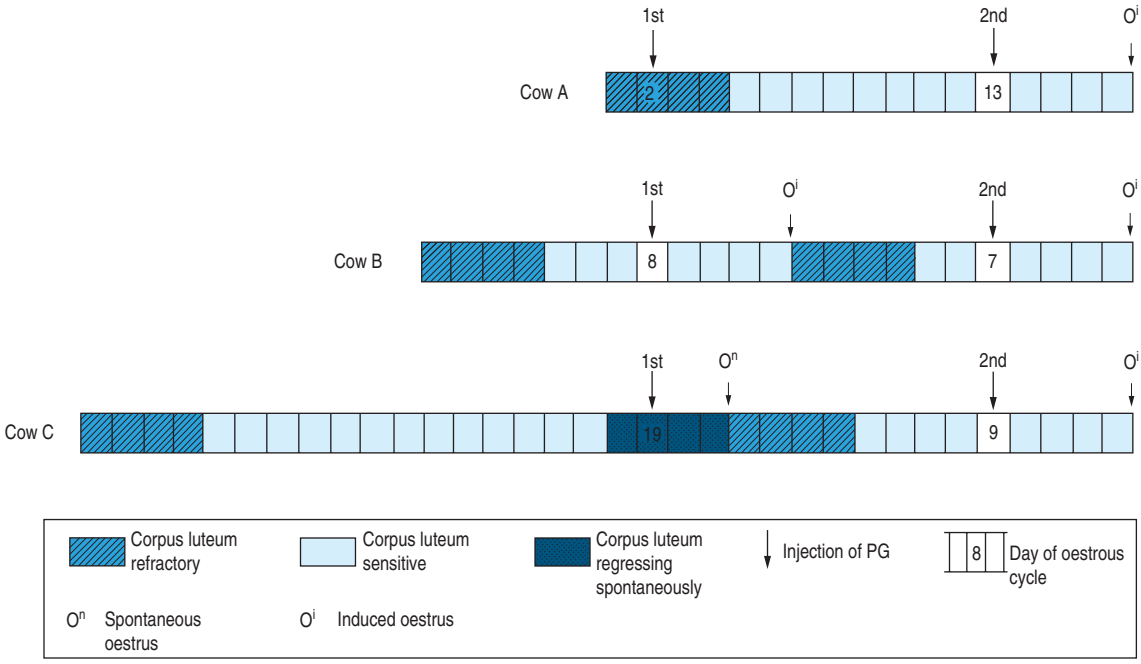

• **Fig. 8.7** Synchronization of oestrus in cows by the administration of two injections of $PGF_{2\alpha}$ or an analogue at an 11-day interval.

double fixed-time AI at 72 and 96 hours or 72 and 90 hours after the second injection will result in conception rates that are similar to those following insemination at a spontaneous oestrus. A single fixed-time insemination (at 84 hours) can be used but will result in reduced conception rates. A mixed programme, using oestrus detection after the first injection and then fixed-time AI after the second, can also be used. There is no evidence of any difference in efficacy between natural $PGF_{2\alpha}$ and synthetic analogues when used in such a programme.

When used on its own, prostaglandin does not produce tight synchronisation; this is because the time between injection and ovulation is dependent on the stage of development of the dominant follicle. This 'follicle effect' is likely to be one of the main reasons why synchronisation is improved by adding progesterone to a prostaglandin programme, as the use of progesterone controls follicular development. The importance of follicle stage in determining the response to prostaglandin was also the key driver behind the development of the Ovsynch programme (covered later in this chapter).

Uses in the Horse

Prostaglandin $F_{2\alpha}$ and its synthetic analogues are the compounds that are most commonly used to manipulate the oestrous cycle in mares (Coffman et al. 2016). If given more than 5 days but less than 14 days after ovulation, a single injection of $PGF_{2\alpha}$ will reliably induce oestrus (on average 3–4 days after treatment). The use of $PGF_{2\alpha}$ enables mares to be mated or artificially inseminated on predetermined days, eliminating the need for the frequent teasing of mares and simplifying travel arrangements for either the mare or the stallion. They can be particularly useful when an oestrus has been missed, especially the 'foal heat', as their use eliminates the need to wait for the next spontaneous heat.

However, as in cattle, the timing of ovulation after an injection of $PGF_{2\alpha}$ is very variable. The crucial factor is the size of the ovulatory follicle as there is an inverse correlation between follicle diameter at the time of treatment and the interval between treatment and ovulation (Burden et al. 2015). For very large follicles (larger than 35 mm), the response depends on the stage of development of the follicle, with most mares with follicles in the growing phase responding to treatment by ovulating around 5 days after treatment, although a small proportion (approximately 18%) will ovulate within 48 hours, often with no observable oestrus behaviour. Mares with atretic follicles will have a prolonged interval to oestrus (up to 15 days), as follicles will regress without ovulation followed by the emergence and, eventually, the ovulation of a new dominant follicle (Burden et al. 2015). It is thus recommended that $PGF_{2\alpha}$ treatment in the mare is accompanied by a veterinary examination pre- and posttreatment to identify the likely response (Coffman et al. 2016).

Uses in the Sheep

Provided there have been more than two days since ovulation, the injection of $PGF_{2\alpha}$ or its analogues will induce oestrus in responsive ewes approximately 36 to 46 hours later (Haresign & Acritopoulou 1978). The timing of ovulation is variable depending on the stage of the dominant follicle (Fierro et al. 2013). As in cattle, the use of a double-dose $PGF_{2\alpha}$programme (with the injections usually given 8–11 days apart) increases the proportion of animals responding and tightens the timing of ovulation compared with a single $PGF_{2\alpha}$ injection at a random stage of the oestrus cycle. Nevertheless, such protocols do not generally result in good reproductive outcomes when used with fixed-time AI (Fierro et al. 2013).

Uses in the Goat

Oestrus can be efficiently synchronised in goats by using two doses of $PGF_{2\alpha}$ (or an analogue) 10 days apart, but the fertility rate is reduced, compared with progesterone-based programmes (Duque-Bonisoli, et al. 2012).

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Uses in the Pig

Reliable synchronisation of oestrus in groups of sows and gilts would have many practical applications, particularly in conjunction with the use of AI to enable batch farrowing to occur. Unfortunately, prostaglandins and their analogues are not luteolytic until the 11th or 12th day of the oestrus cycle. Devising a regimen of prostaglandin injections that will synchronise groups of sows is therefore not possible.

Uses in the Dog and Cat

Prostaglandins do not readily cause luteolysis in these species.

GnRH and Its Analogues

GnRH, synthesised within the cells of the hypothalamus, is the hormone responsible for controlling the release of the anterior pituitary hormones. The development of artificial synthetic GnRH in the early 1970s and then the development of its synthetic analogues, e.g., buserelin, led to multiple studies in many species of the efficacy of GnRH and its analogues in controlling reproduction. In cattle GnRH (and analogues) have been used to treat cystic ovarian disease, delay luteolysis, and stimulate ovulation; however, their main use currently is as an integral part of synchronisation programmes designed to control the timing of ovulation, such as Ovsynch (Peters 2005). In small ruminants GnRH has been used much less commonly although it is effective at stimulating ovulation and has been shown to delay luteolysis (Newcombe & Peters 2014). In pigs there has been limited use of GnRH, though studies have shown that, especially when used after equine chorionic gonadotrophin (eCG) treatment, it can induce premature puberty in gilts, and it may also increase fetal survival when used during early pregnancy (Drost & Thatcher 1992, Newcombe & Peters 2014).

In mares GnRH does not stimulate ovulation in animals in deep anoestrus or in those with regressing follicles. However, cyclic mares with mature follicles or mares in the transitional period are more responsive, although this response, particularly in the latter, is less reliable than in cattle (Newcombe & Peters 2014) and requires significantly higher or repeated doses (Newcombe & Cuervo-Arango 2017). The need for repeated doses led to the development of implants, which release GnRH agonists (such as deslorelin). These are effective (over 90% of mares ovulate within 48 hours of administration) but expensive and, if used continuously, can result in suppression of FSH and LH secretion, with extension of the interoestrus interval (Newcombe & Cuervo-Arango 2017). This suppressive effect means that deslorelin implants can be used to suppress cyclical ovarian activity and testicular function, with commercial products currently available to suppress fertility for 12 months per implant in the male dog and ferret.

Compounds That Replace or Supplement Pituitary Gonadotrophins

Commercial products based on purified pituitary extracts are available that contain FSH and LH, but these are relatively expensive; thus in the majority of cases alternative hormones with similar activities are used rather than the specific gonadotrophins.

In domestic animals two substitutes are commonly used: (1) eCG, obtained from the serum of pregnant mares (see Chapter 5), which has a mainly FSH-like effect but also has some LH-like activity; and (2) human chorionic gonadotrophin (hCG), obtained from the urine of pregnant women, which has mainly an LH-like effect but with some FSH-like activity.

In pigs eCG has been used to induce oestrus after weaning and to stimulate puberty in gilts and oestrus in anoestrus sows. As eCG stimulates oestrus rather than ovulation, particularly in anoestrus sows and gilts, better results are achieved if eCG is used in combination with a product that specifically stimulates ovulation such as GnRH, hCG or porcine LH (Kirkwood & De Rensis 2016).

In cattle eCG treatment has been used to stimulate follicular development and ovulation. Large doses (1500–3000 iu) are used in embryo transfer programmes to induce superovulation; smaller doses are used in multihormone synchronisation programmes to increase follicle size and vitality at insemination. On its own, hCG has generally been used in cattle as an alternative to GnRH.

In ewes and does the use of eCG alone to induce oestrus in seasonally anoestrous ewes is not very successful, but if progesterone is administered to the ewes before the injection of eCG, then there is synchronised oestrus and ovulation. However, attempts to stimulate an early return to cyclical activity in lactating ewes have proved to be difficult, particularly in those that are lactating heavily. As in cattle, high doses of eCG have been used to produce superovulation.

In queen cats hCG has been used to induce ovulation (as an alternative to using a vasectomised tom or simulating coitus by swabbing the vagina). Ovulation stimulated in this way leads to pseudopregnancy and prevents the return to oestrus for 4 to 8 weeks.

Combinations of eCG/hCG have been used to induce oestrus in the anoestrous bitch, sometimes in combination with oestrogens. The induction of a behavioural response has usually been good, but the numbers of bitches that ovulated and subsequently conceived has usually been poor.

Surprisingly, eCG does not appear to stimulate ovarian activity when given to mares in winter anoestrus. The reasons for this are probably twofold: first, it may be that the dose required to stimulate follicular development is large; and second, it is likely that eCG alone is not responsible for stimulating the wave of accessory follicles during early pregnancy.

Hormone Combinations

Uses in Cattle

Oestrus, and thus ovulation, cannot be synchronised very closely using a simple synchronisation regimen such as prostaglandins or progestagens alone (Coulson et al. 1979). This can result in poor conception rates when fixed-time AI is used, especially in cows rather than nulliparous heifers (Pursley et al. 1997). The two key reasons for this failure are: first, the variability in the refractory period of the CL after ovulation to luteolytics such as $PGF_{2\alpha}$; and second, the complexity and variability of the follicular wave patterns that occur in cattle. If, for example, $PGF_{2\alpha}$ is given to a cow with a newly emergent dominant follicle, then ovulation will occur later than if it is given to a cow with an older dominant follicle. This led to the development of the 'Ovsynch' protocol that aimed to synchronise follicular waves so that ovulation occurred over a short enough period of time that a single fixed-time AI could produce reasonable conception rates (Pursley et al. 1995).

In the Ovsynch programme, GnRH is given on day 0, followed by $PGF_{2\alpha}$ on day 7, with a second GnRH 56 to 60 hours later (day 9) and a single fixed-time AI 16 to 24 hours after the second GnRH injection (Fig. 8.8). It is the GnRH injections that synchronise the follicular waves of the treated cows. In responsive cattle, i.e., those with LH receptors on dominant follicles (usually

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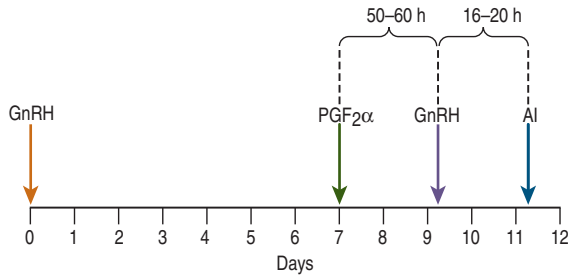
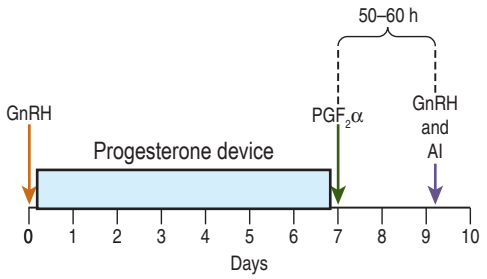


• **Fig. 8.8** Protocol used for oestrus-synchronization in cows using a combination of GnRH and Prostaglandin $F_{2\alpha}$ followed by a single fixed-time AI (Ovsynch programme).



• **Fig. 8.9** Protocol used for oestrus-synchronization in the heifer using a PRID or CIDR together with a combination of GnRH and Prostaglandin $F_{2\alpha}$ followed by a single fixed-time AI.

8 mm or larger in diameter), the first dose of GnRH will either induce ovulation or atresia of the dominant follicle, enabling the emergence of a new follicular wave approximately 1 to 2 days later. Nonresponsive cattle will generally have early stage follicles (fewer than 4 days), so even if nonresponders are included, the range of ages of the dominant follicles present when the $PGF_{2\alpha}$ is given on day 7 is significantly less than it would have been without the GnRH, with most having a dominant follicle that is capable of ovulating and producing a fertile egg. The second injection of GnRH brings the follicular dynamics of the treated cows even closer together, as it stimulates the preovulatory LH surge so that ovulation in the treated group is concentrated over a shorter period of time. This manipulation of the follicular waves means that a single fixed-time AI can be used, so that, even though the Ovsynch programme decouples oestrus behaviour from ovulation, this is not important as the programme does not depend on oestrus detection to be successful.

The programme is most successful in lactating cows; it is less successful in cows in anoestrus and, particularly, in nulliparous heifers. These cows need different programmes. However, even for lactating cows, there are still issues with the programme, with in some cases 40% of cows failing to respond to treatment (Martins & Pursley 2016). The key issues seem to be: (1) insufficient synchronisation of follicular waves; and (2) failure of luteolysis.

In the first case the problem is the cows that have dominant follicles that do not respond to the first GnRH, especially those that are about 3 to 4 days of age at the time of the injection. These dominant follicles may undergo atresia prior to the injection of $PGF_{2\alpha}$; if this occurs, the new dominant follicle will be too immature to respond to the second GnRH injection. The solution to this issue is to presynchronise the treated cows so that they start the Ovsynch programme in days 5 to 9 of the oestrous cycle (Vasconcelos et al. 1999). Presynchronisation programmes are now commonly used in the US prior to the Ovsynch programme. An example of such a programme is Presynch-10, in which two injections of $PGF_{2\alpha}$ are given 14 days apart, with the second injection 10 days before the first GnRH of the Ovsynch programme (Martins et al. 2017). However, Presynch-10 is only one of a large number of presynchronisation programmes that have been tried in order to further tighten the synchronisation of cows before they enter the Ovsynch program. One example is CIDR-PS (Rutigliano et al. 2008), in which cattle receive a 'progesterone insert' for 7 days, with removal and a $PGF_{2\alpha}$ injection 3 days before the start of the Ovsynch programme; other programmes include a double Ovsynch programme (with the first programme ending 7 days before the second) and the G6G programme ($PGF_{2\alpha}$ followed by GnRH 8 and 6 days before the Ovsynch programme, respectively) (Martins & Pursley 2016). Most of the tested presynchronisation programmes do increase the proportion of treated cows that become pregnant after the Ovsynch programme. However, for the more complex programmes the improvements in fertility probably do not cover the costs of the presynchronisation programme (i.e., the costs of handling in addition to the costs of the pharmaceuticals), especially when compared with the simpler programmes such as Presynch-10.

The other key issue is luteolysis. Failure to achieve complete luteolysis after the $PGF_{2\alpha}$ injection is strongly associated with failure to become pregnant after an Ovsynch programme. The proportion of cows in which luteolysis is not complete seems to be around 10% to 11%, and can be significantly reduced by including a second $PGF_{2\alpha}$ injection 24 hours after the first (Martins & Pursley 2016).

Synchronisation in Heifers

Ovsynch-type programmes significantly reduce the conception rate in nonlactating heifers compared with insemination after observed oestrus (Martins & Pursley 2016). So to synchronise heifers, alternative programmes are needed. McDougall et al. (2013) showed that in heifers at pasture, the combination of an intravaginal progesterone device with an Ovsynch-like programme, resulted in higher conception rates after fixed-time AI than insemination after observed oestrus produced in response to a $PGF_{2\alpha}$ injection (57% vs 48%, respectively), but only when the heifers were inseminated on day 9 of the programme alongside the second GnRH injection ('Cosynch'; Fig. 8.9). Cosynch programmes (with or without progesterone) have the advantage of less handling than Ovsynch programmes and so are commonly used in situations in which handling is an issue, such as in lactating beef cattle, even though using a Cosynch rather than an Ovsynch programme may reduce conception rates by 3% to 4% (Colazo & Mapletoft 2014).

However, Sahu et al. (2015) compared the Cosynch programme recommended by McDougall et al. (2013) with fixed-time AI after a standard 7-day progesterone programme with $PGF_{2\alpha}$ on day 6 (P+PG) and found no difference in conception rates between the two programmes (52.4% and 54.8%, respectively), despite the P+PG programme being significantly cheaper. However, P+PG did require four handlings rather than the three required by the Cosynch program, so Sahu et al. (2015) concluded that the choice of programme should be made at an individual farm level and take into account labour costs as well as pharmaceutical costs.

Synchronisation in Anoestrus Cows

In anoestrus cows the Ovsynch programme is much less effective than in cycling cows because of the lack of response of any of the follicles to the first GnRH injection, the low progesterone concentrations on day 7 (partly because of the lack of response to GnRH

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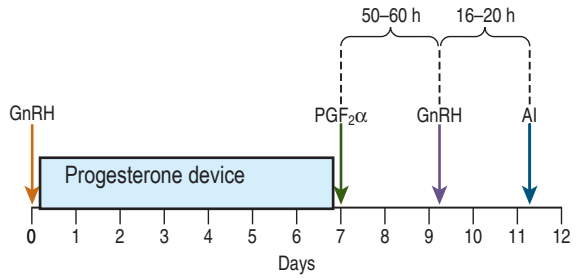

• **Fig. 8.10** Protocol used for oestrus-synchronization in the anoestrus cow using a PRID or CIDR together with a combination of GnRH and Prostaglandin $F_{2\alpha}$ followed by a single fixed-time AI.

but also because cows anoestrus will not have and endogenous CL), and the high risk of a short luteal interval even if ovulation is synchronised. Some presynchronisation programmes, e.g., G6G, have been shown to improve the response of anoestrus cows to the subsequent Ovsynch programme (Colazo & Mapletoft 2014), but probably the most commonly used programme to treat anoestrus cows is an Ovsynch programme with the addition of intravaginal progesterone device between Day 0 and Day 7 (Fig. 8.10). McDougall (2010) compared the Ovsynch programme alone to an Ovsynch programme with additional progesterone (O+P4) in pasture-based cattle that had not shown oestrus prior to 10 days before the start of the spring breeding season. They reported that, although both Ovsynch and O+P4 resulted in shorter intervals from the start of treatment to conception compared with untreated animals, the addition of progesterone decreased these intervals by a further 6 days compared with Ovsynch alone. In this study 73% of the cows did not have a detectable CL at the start of the synchronisation program, so this large controlled study of over 2000 cows confirms the value of using progesterone routinely in Ovsynch-based synchronisation programmes in which anoestrus is a significant problem, especially as even in cows with a visible CL on day 0, O+P4 resulted in a significantly shorter median interval to conception than Ovsynch alone.

The use of eCG at the same time as $PGF_{2\alpha}$ stimulates the growth of the dominant follicle. This effect has been shown to be beneficial alongside O+P4 programmes in anoestrus cattle, especially when the body condition of treated cattle is not optimal, as well as in *Bos indicus* cattle (Bó et al. 2013).

Hormone Combinations in Other Species

Of the other domestic species, hormone combinations are commonly used only in the doe and ewe. As discussed previously, the most commonly used regime is a combination of eCG and progesterone (or its analogues). However, $PGF_{2\alpha}$ is often added to an eCG/progesterone programme (either at the beginning or the end of the programme) to remove any active CLs.

Other Hormones

In bitches prolactin plays a role in determining the length of the interoestrus interval. The exact mode of action is unknown but may be related to an effect on gonadotrophin secretion and/or ovarian responsiveness to gonadotrophins. Prolactin inhibitors such as cabergoline and bromocriptine can be used to induce a fertile oestrus with normal pregnancy rates in anoestrus bitches. Results are generally better in bitches in midoestrus than those in early oestrus.

Immunological Methods

Immunocontraception

Immunocontraception, in which animals are vaccinated against specific proteins that have a crucial role in the normal functioning of the reproductive cycle, has the potential to be a significant tool in the management of reproduction in wild and domestic animals. The two most commonly targeted proteins have been the zona pellucida proteins and GnRH (Naz & Saver 2016). Zona pellucida vaccines have been shown to be efficacious in a wide range of wild and domestic mammals, from brushtail possums to elephants to wild and domesticated ungulates. Their efficacy in carnivores, including dogs and cats, is limited.

Although zona pellucida vaccines have been used widely, particularly in free ranging or wild animals, anti-GnRH vaccines are used far more commonly in commercial situations. In cows a standard two dose course of an anti-GnRH vaccine has been shown to induce a reversible suppression of oestrus activity for more than two months, whereas in horses efficacy for at least three months has been demonstrated. One advantage of GnRH-based vaccines over zona pellucida vaccines is that the former can be used in both males and females; vaccination reduces sexual and aggressive behaviour so can be a useful tool in managing groups of entire males.

Antiandrostenedione

Vaccination has also been used to increase fertility. Vaccines produced by conjugating a derivative of the natural ovarian hormone androstenedione with human serum albumen have been shown to significantly increase the number of lambs born and reared (usually by about 20%). This effect is mediated solely by an increase in the ovulation rate (Juengel et al. 2013). The initial vaccination protocol requires two doses four weeks apart, but only a single vaccination is required in the following season; using this vaccination procedure requires careful management. Mating must start at least 25 days after the final vaccination, as otherwise there may be detrimental effects on conception rate and early embryo development. Treated ewes must be fed adequately throughout pregnancy because of the dangers of pregnancy toxaemia occurring.

Many other compounds have been identified as being potential targets for the use of vaccines to modify reproductive function. For example, vaccination against bone morphogenetic protein has been shown to increase ovulation rate in ewes, but not in lambs born or weaned (Juengel et al. 2013). Perhaps the most common target for such vaccines has been against inhibin, in which vaccination has been shown in females to increase FSH concentrations and ovulation rates as well as promoting follicle development; in males it has been used to increase sperm production and advance the onset of puberty (Zhu et al. 2013).

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PART 2

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9

Abnormalities of Development and Pregnancy

PETER WINDSOR

Introduction

The demand for improved understanding of the causes of abnormalities that occur during development in pregnancy, particularly those presenting as embryonic, fetal, or congenital malformations in human and other animal populations, is growing. There is increasing concern that teratogenic agents, including chemical toxicities, deficiencies, infections, and physical conditions such as heat, can alter fetal morphology or subsequent function. The embryo is most susceptible to teratogenic agents during periods of rapid differentiation, with the stage of development of the embryo determining the susceptibility to teratogens. The most critical period in the development of an embryo or in the growth of a particular organ is during the time of most rapid cell division.

There is also a growing need to distinguish abnormalities due to teratogens from genetic disorders affecting embryonic development or, more recently, to identify where genetic-environmental interactions are occurring. This has led to an increasingly vast amount of literature on the abnormalities of development and pregnancy. This is to be expected as reports of investigations of losses of pregnancy and of congenital disorders arising from naturally occurring and induced insults to embryonic development through teratogenicity in laboratory, farm animals, and, occasionally, companion animals may offer important comparative insights into clinical medicine and greatly assist understanding of the pathogenesis of congenital disorders.

Many chemical agents have been subjected to investigation for detrimental effects on the embryo or fetus during pregnancy; including cosmetics, food additives, fungicides, herbicides, insecticides, pesticides, and other agricultural chemicals, as well as environmental contaminants, drugs, hormones, and vaccines. These investigations have often been retrospective, occurring well after the emergence of a new syndrome of malformation has been associated with a previously unrecognised teratogen. With the growing history of the numerous environmental pollutants capable of causing teratogenic effects, it is surprising that protocols for determining teratogenicity are still arbitrary. Binding international agreements are required to prohibit the distribution and sale of teratogenic compounds (McGeady et al. 2017).

To investigate emerging syndromes of teratogenicity, an extensive knowledge of the recorded occurrences of losses in pregnancy and malformations in the various animal species is useful. This is important in farm animals in which losses in pregnancy causes reduced production efficiency and economic losses to producers, including the reputational losses to breeders when congenital malformations are suspected or confirmed as inherited. Knowledge of the aetiology of developmental disorders has become essential to enable breeders to manage the hazards causing losses in pregnancy and from congenital disease. Increasingly, genetic disease information is readily available on breed society websites to enable breeding management that avoids the mating of animals that are heterozygous carriers of the same mutation. Information is also increasingly available that enables farm animal managers to minimise the risks of exposure to teratogenic agents during pregnancy. In Australia, the National Arbovirus Monitoring Program regularly updates maps of places in which sentinel herd activity has identified seroconversion to potentially pathogenical arboviruses spread by insect vectors (https://www.animalhealthaustralia.com.au/our-publications/namp-report). This enables the livestock industries to demonstrate that the Bluetongue virus only occurs where few sheep are raised and assists producers to understand the importance of avoiding the movement of pregnant naïve animals into endemically infected zones where Akabane virus infection transmitted by *Culicoides* spp. threatens the embryo and fetus.

Developmental Disorders in Humans

Understanding congenital disorders in human populations associated with familial risk factors causing inborn errors of metabolism has been of importance for many generations of families. The database of human disorders transmitted by genetic factors, Online Mendelian Inheritance in Man (OMIM; https://www.omim.org), is now 50 years old and continues to expand rapidly, with mapping of the human genome enabling more accurate definition and recording of the mutant genes contributing to abnormal phenotypes, particularly the genetic defects that present at birth as congenital deformities. Further, the identification of chemical teratogens causing toxin-induced congenital disorders is well known because the notorious sedative thalidomide (α-phthalimidoglutarimide) was used widely as a sleeping pill and for the treatment of hyperemesis gravidarum, leading to a disastrous epidemic of phocomelia and other malformations (Lenz 1962). Studies in laboratory animals identified wide species differences in teratological susceptibility, with the characteristic limb malformations induced by thalidomide in humans only reproduced in rabbits. Significant differences within species were also demonstrated. Degenerative changes in the neurons and axons of dorsal root ganglia in 13-day-old rabbit embryos from mothers receiving thalidomide in doses of 150 to

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250 mg/kg/day on Days 8 to 12 of gestation suggested the abnormalities of the developing human fetal skeleton were caused by interference with the developing neural crest (neurulation). These lesions in sensory neural tissue were considered to induce a decrease in cell division before the onset of the limb bud outgrowth, at 10 days postcoitum (McBride et al. 1982). Since then, definition of deficiency syndromes causing congenital disorders in humans has advanced, for example, the response of increasing dietary folic acid to reduce risk of spina bifida. Recently, there has been an increase in appreciation of teratogenic viruses afflicting humans after the emergence of Zika virus causing abnormalities of fetal central nervous system (Kim & Shresta 2016).

Developmental Disorders in Animals

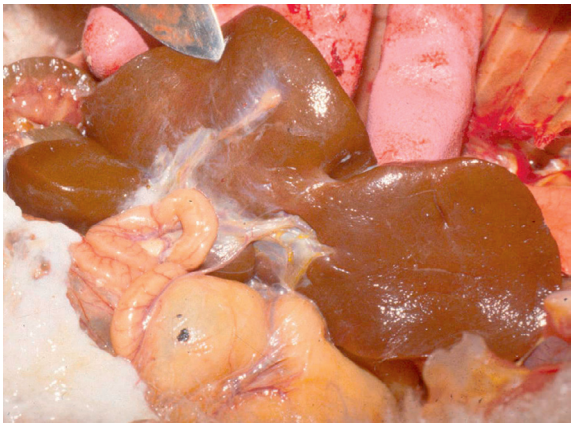
Veterinarians and animal scientists have also been managing abnormalities of pregnancy for many generations. Inbreeding is common in domestic animal breeding, particularly when artificial reproductive techniques have enabled vast numbers of progeny to be descendants to individual elite sires and their immediate progeny (Windsor & Agerholm 2009). Further, in some locations, embryonic and fetal infections of animals with teratogenic viruses or toxins, plus deficiency syndromes causing congenital disorders, occur regularly and are increasingly well described. However, obtaining an accurate diagnosis and determining the cause of losses in pregnancy or an abnormality of fetal development in an animal remain challenging for veterinarians and diagnosticians. This reflects both the rapidly expanding knowledge on the causes of the 'known disorders', plus the likelihood that unreported syndromes and the inevitability that 'newly emerging disorders' will continue to occur and demand attention and management. Importantly, few of these emerging disorders present initially with diagnostically characteristic or pathognomonic clinical signs and pathological lesions and may be readily confused with the known disorders (Agerholm et al. 2015). As differentiation of the various entities presented to veterinary diagnosticians can be challenging, particularly when the affected animal appears to be afflicted by an unfamiliar disorder, this chapter reviews some of the current and emerging knowledge of fetal developmental disorders in animals, particularly in livestock.

Aetiologies of Disorders of Development

Mammalian embryonic development is complex, involving delicate synchronisation of numerous events. Aberrations can potentially occur during gametogenesis, fertilisation, blastogenesis, embryogenesis, or in vulnerable periods during fetal development and growth. Historical estimates of the known causes of human congenital malformations suggested that only about 30% to 35% of the human congenital malformations have clearly identifiable aetiologies: about 20% genetic; 5% to 6% chromosomal; 4% to 5% maternal diseases; and 2% to 3% caused by drugs and environmental chemicals. The causes of a majority (65%–70%) of human congenital disorders remain unknown or are potentially caused by multifactorial interactions between genetic and environmental factors, representing complex host–pathogen–environment interactions occurring in the pathogenesis of disease.

Similar complexities occur in the study of losses in pregnancy and congenital disorders in animals. Identification of a single, predominant causative factor in an animal before or at birth, which is very likely to reflect a detrimental event that occurred much earlier *in utero,* is challenging as aberrations occurring during gametogenesis, fertilisation, blastogenesis, and embryogenesis that result in mortalities are usually only recognised as reproduction failure. However, lethal insults of fetal development later in gestation may present as abortions, fetal mummification, stillbirth, or neonatal mortality. Nonlethal aberrations may lead to lowered viability at birth, possibly accompanied by congenital abnormalities with grossly visible lesions that may suggest damage occurring during a particularly vulnerable period of fetal development and growth. It is now well understood that infection of a pregnant ruminant with a teratogenic arbovirus induces various lesions of the developing fetal nervous system once the fetus has commenced development of an immunological capacity at the end of the first trimester. The lesions produced are dependent on the stage of development of the fetus. For example, in cattle in Australia, Akabane virus (AKAV) induces hydranencephaly at the end of the first trimester, followed by arthrogryposis in midgestation, whereas bovine viral diarrhoea virus (BVDV) may induce cerebellar dysplasia from infection of the CNS in midgestation (Kirkland et al. 1988). However, if the agent only induces harm in the developing embryo when aligned with maternal nutritional deprivation or excess or some other maternal physiological disturbance, then identifying the aetiology of the loss or congenital disorder may become very complicated. An example was the suspected association of the plant *Dysphania glomerulifera* as an intoxication of sheep after drought in parts of Australia, leading to neonatal biliary atresia (Fig. 9.1) that required several decades of research before it could be confirmed that this plant was the source of a unique toxin involved in inhibiting posthepatic biliary development *in utero* (Harper et al. 1990, Koo et al. 2015).

Potentially, the simultaneous ingestion or administration of certain trace elements and a wide variety of chemical substances in food additives, fertilisers, fungicides, herbicides, and other chemicals used in agriculture can be problematic. Chemicals and therapeutics may well be shown not to be teratogenic when used separately (enabling their registration by regulatory agencies for the use intended), yet concurrent or inappropriate use of these agents may induce potentiating or synergistic effects with nonchemical environmental factors. This may make definitive identification of the primary aetiology extremely difficult. Further, physical agents, including maternal infections and noninfectious maternal disorders, hypoxia, hypothermia, hyperthermia, maternal immobilisation, seasonal variations in the occurrence of infections, and available dietary components, all potentially increase this complexity. These numerous and variable risk factors for developmental and congenital



• **Fig. 9.1** Absence of the gall bladder in a neonatal lamb with biliary atresia after grazing of the dam on *Dysphania glomerulifera* containing biliatresone.

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disorders, particularly in farm animals, requires that investigators possess considerable knowledge of the genetic and chromosomal, nutritional and dietary, physical, chemical, hormonal, viral, and other agents that can induce teratogenic pathogenical pathways in the embryo and fetus.

Investigating Disorders of Development

The first questions asked of a veterinarian by an owner of an animal that has lost a pregnancy or given birth to animal with a congenital disorder is usually 'what's caused this'. Owners are usually then interested in asking 'did this animal become infected or was it exposed to something that caused this loss, or is it affected by a known inherited disease (that has been recognised in this particular species and breed)'.

Answering the second part of this question on inherited disorders is facilitated by regularly examining the database of animal disorders similar to OMIM: Online Mendelian Inheritance in Animals (OMIA; http://omia.angis.org.au/). OMIA readily assists the process of reviewing known genotypes and phenotypes associated with inherited disorders, with convenient examination of the published literature of those disorders in which a referenced publication in a reputable journal has occurred. The increasing availability of molecular approaches that may be used for mutation detection, including sequencing of candidate genes, single nucleotide polymorphism array with genome wide association studies, and exome or whole genome sequencing, has greatly facilitated the recognition of disease-causing mutations in animals. As of early December 2017, OMIA listed 3372 disorders in animals, of which 1344 are Mendelian traits. Importantly, 741 have a disease-causing mutation identified; this includes: 144 of 243 Mendelian disorders of cattle; 49 of 102 disorders of sheep; 10 of 16 disorders of goats; 28 of 68 disorders of pigs; 234 of 306 disorders of dogs; and 62 or 95 disorders in cats. Several of the more important inherited developmental disorders in cattle and the mutations described are listed (Table 9.1).

Providing an answer to the first part of the question 'is this animal affected by a toxic plant, nutritional deficiency or excess, or an infectious agent that damaged the development of the embryo or fetus during gestation' involves consideration of epidemiological risk factors, including whether the animals are located in areas where infectious agents, teratogenic plants, or nutritional aberrations may occur. This requires diligent examination of all of the environmental conditions (e.g., plants, feeds, soil, water, housing, treatments, etc.) that occurred during the gestation period. Investigating embryonic and fetal losses due to infections requires extensive investigative protocols and usually requires laboratory tests aimed at gathering evidence of inclusion or elimination of the known infectious agents causing losses in that species.

Preferably, these investigations are assisted by information on breeding occurrence (numbers of females successfully mated), pregnancy status (numbers of females successfully maintaining pregnancy until the time of pregnancy testing), neonatal animals delivered, and marking (for initial interventions, including application of identification devices and health products including vaccination) and weaning rates. The calculation of these various rates of viable offspring in the population at each stage in the reproductive cycle enables identification of the stage at which the losses occurred. Knowledge of the differential diagnostic list for losses at that stage of the cycle then enables a more informed investigation.

The investigative process is greatly enhanced by collection of a fetus and/or placenta or, if available, several of these most valuable of specimens. Laboratory examinations for the presence of infectious agents can occur by culture of the pathogen or detection of antigens or PCR products. Furthermore, collection of presuckle serum or body cavity fluid from the fetus or affected neonates and serum from the dam enables laboratory examination for evidence that a teratogenic pathogen was formerly present and has produced a pathogen-specific antibody response or at least an elevation in nonspecific immunoglobulin (e.g., IgG), suggesting an infectious process occurred before birth. Extending this examination to include herd seroprofiling can be useful, enabling comparison

TABLE 9.1 Some inherited disorders of cattle with mutations and chromosomal location identified

Case Definition	Lesion	Breed	Associated Gene(s)	Chromosome
Alpha-mannosidosis	CNS lysosomal storage	Angus	MAN2B1	7
Arthrogryposis multiplex	Limb fixation	Angus	ISG15, HES4, AGRN	12
Brachyspina	Shortened spine, other	Holstein–Friesian	FANCI	21
Bulldog dwarfism	Severe chondrodysplasia	Dexter	ACAN	21
Citrullinaemia	CNS cerebrocortical oedema	Holstein–Friesian	ASS1	11
Congenital Myoclonus	Fractured hips	Poll Hereford	GLRA1	7
Complex veterbral malformation	Shortened spine, limb fixation	Holstein–Friesian	SLC35A3	3
Contractural arachnodactyly	Hindlimb muscle dystrophy	Angus	ADAMTSL3	21
Developmental duplications	Polymelia, others	Angus	NHLRC2	26
Disproportionate dwarfism	Mild chondrodysplasia	Angus	PRKG2	6
Maple syrup urine disease	CNS myelin oedema	Poll Hereford	BCKDH	18

Data from OMIA.

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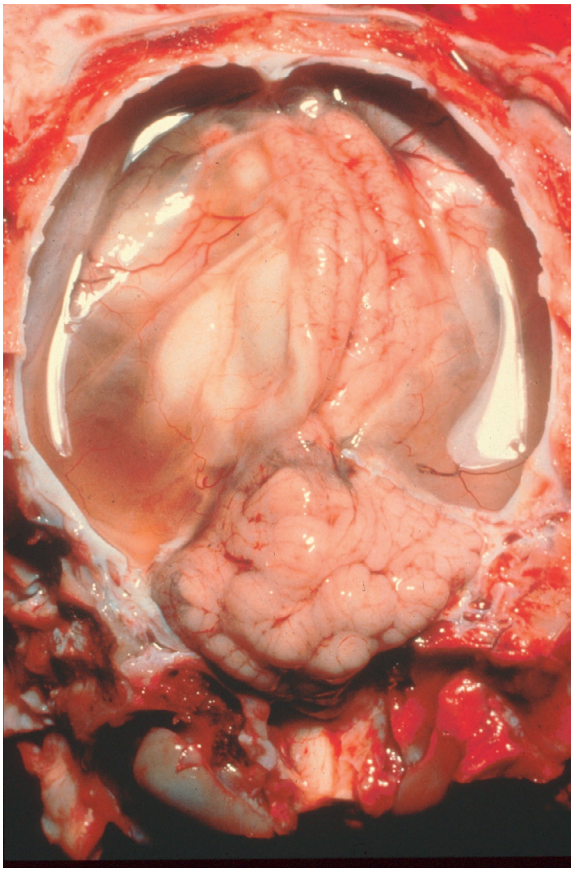
TABLE 9.2 Teratogenic lesions in calves after common intrauterine viral infections

Lesion Definition	BVDV	AKAV/AV	SBV	BTV
Hydranencephaly	✓	✓	✓	✓
Porencephaly	✓	✓	✓	✓
Hydrocephalus	✓	rarely	✓	✓
Microencephaly	✓	✓	✓	✓
Microphthalmia	✓	–	–	–
Cerebellar dysplasia	✓	rarely	✓	✓
Arthrogryposis	–	✓	✓	–
Spinal deformity	–	rarely	✓	–

BVDV: Bovine Viral Diarrhoea virus, AKAV/AV: Akabane & Aino viruses, SBV: Schmallenberg virus, BTV: Bluetongue virus
Adapted from Agerholm et al. 2015.



• **Fig. 9.2** Hydranencephaly in neonatal 'dummy calf' after birth to a cow infected with Akabane virus at between Days 80 to 105 of gestation.

of the serological status of females that were 'affected' (i.e., lost a fetus) versus 'unaffected'. This enables definition of an 'at risk' population of animals with evidence for 'inclusion' or 'exclusion' of a pathogen in association with the problem and ensuring the investigation can proceed in a more systematic manner. For arbovirus infections, viral serological and insect distributions should be regularly consulted. Movements of arthropod vectors from known endemic areas are dynamic, as is the risk of exposure of the population to an arbovirus infection *in utero* (NAMP; https://namp.animalhealthaustralia.com.au/public). Infection of ruminants by the arboviruses, AKAV or Aino virus (AV) and BTV, plus Schmallenberg virus (SBV) in Europe, causes a range of congenital malformations in the fetus and neonatal calf, depending on the stage of fetal neural development when the dam is infected (Table 9.2). A complication of these investigations is that, in most cattle populations, BVDV remains endemic and is capable of causing an even wider range of congenital malformations in the fetus and neonatal calf over a longer period of the gestation. For investigation of potential teratogenic plants, accessing local veterinary knowledge and use of compiled knowledge resources (McKenzie 2012) can assist this process.

Despite widely available resources and informed local knowledge, diagnosing developmental disorders on the basis of gross morphology of the defective offspring often remains challenging. This is particularly problematic when determining whether the disorder is not due to a noninfectious cause. Knowledge of the genetic defects that cause similar lesions to teratogenic viruses, or so-called 'phenocopies', even in cases in which viral antigen or antibodies may be present in an animal, is required. In cattle and sheep consideration of any likelihood of exposure of the fetus in midgestation to an animal that is persistently infected (PI) with pestivirus, i.e., in cattle BVDV and in sheep ovine border disease virus, is advised.

An understanding of the timelines that occur *in utero* when the fetal neuromuscular system is most vulnerable to a teratogenic virus is important, as the pathology that occurs after infection is determined by the stage of gestation at the time of fetal infection. For example, in cattle, intrauterine infection of the fetus by BVDV, AKAV/AV, SBV and BTV during the susceptible periods of development (Days 60–180 of gestation) may cause malformations in the central nervous system. Lesions in the brain of AKAV/AV occur from infection at Days 85 to 105 of gestation when, typically, hydranencephaly (Fig. 9.2) occurs. This may be accompanied by porencephaly and even secondary hydrocephalus (with or without the Arnold-Chiari malformation that occurs from progression of the cerebellum into the foramen magnum) (Fig. 9.3). Calves with cerebellar dysplasia from BVDV have typically been infected at around 150 days of gestation (Fig. 9.4) and need to be investigated for pestivirus (although numerous abnormalities are possible with BVDV, including porencephaly). Lesions in the spinal cord typically cause arthrogryposis when infection with the Simbu viruses (most commonly AKAV) at 120 to 160 days (Fig. 9.5), resulting in malformations of the axial and appendicular skeleton (Table 9.2). Interestingly, the endemic presence of these teratogenic viruses may delay the initial diagnosis of emerging inherited 'phenocopies' of these disorders. This situation occurred with arthrogryposis multiplex (OMIA 001465) in Angus calves, at least in eastern Australia, where an inherited disorder that presented as cases of arthrogryposis was attributed for several years to AKAV infection. For consideration of involvement of plant toxins as a cause of the disorder, knowledge of the epidemiology of and potential access of the pregnant dam to known potential teratogenic plants during the first trimester of gestation (e.g., *Verratrum californicum, Conium maculatum, Nicotiana glauca,* or grazing lupins or sorghum) is advised.

When conducting an investigation of a suspected inherited disorder, consideration of pedigree information and whether parentage verification (e.g., by blood typing or molecular methods) is required plus potential mode of transmission is advised

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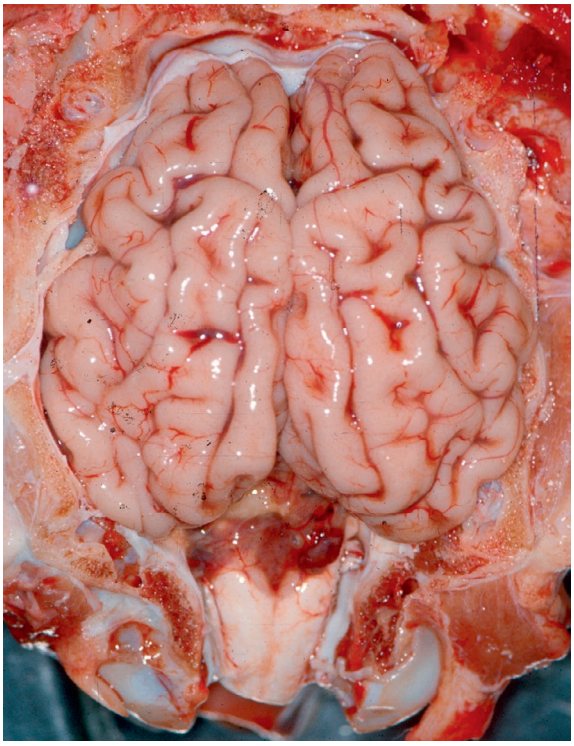
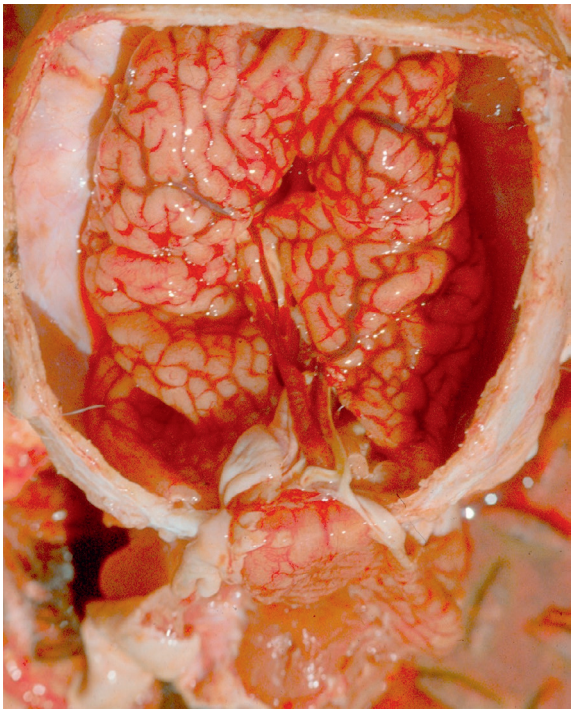


• **Fig. 9.3** Hydrocephalus in a calf with posterior prolapse of the cerebellum causing Arnold-Chiari malformation.



• **Fig. 9.4** Cerebellar dysplasia in a severely ataxic calf after birth to a cow infected with BVD virus at about Day 150 of gestation.

(e.g., autosomal recessive disorders require both dam and sire to be heterozygous for the suspected defect). For flocks and herds that have consistently used registered sires, a simple pedigree analysis to determine whether familial relationships exist between the affected animals and their parents should be undertaken if sufficient cases

• **Fig. 9.5** Athrogryposis in a calf with fixation of the limbs born to a dam infected with Akabane virus at between Days 120 to 160 of gestation.

are available. This may enable a more rational and less expensive approach to laboratory investigations of nongenetic causes, as it may quickly become apparent that all the affected progeny have the same or closely related sire(s) and the dams of affected progeny are also related to the sire(s). DNA parentage analysis can also be used to determine whether more than one sire is involved. The important question to consider is whether the affected phenotype is present in the progeny of a particular animal at a prevalence that is greater than would be likely by chance, after considering other factors. Confusion can occur, for example, when one single-sire mating group is bred in the only paddock with a teratogenic plant, or at the time of year when insect vectors capable of transmitting teratogenic viruses were active, or in cattle and sheep when an animal persistently infected with BVDV or OBDV was in the vicinity.

With various pathogenical modalities in the genesis of embryonic and fetal losses or congenital disorders, obtaining the evidence to provide accurate diagnoses of the causes of abnormalities that occurred earlier in the developmental processes is one of the most challenging investigative procedures for veterinary practitioners, diagnosticians and researchers to undertake. However, as an understanding of congenital disorders in animals may have important scientific implications both as clues to the mechanisms of pathophysiology and as animal models of human disorders (e.g., biliatresone from *Dysphania spp.* plants in sheep as a model of toxic biliary atresia in infants), it is important that diligent surveillance for congenital disorders, accompanied by systematic investigations, be encouraged. A more rigorous approach to investigating developmental disorders has become particularly important because of the widespread adoption of international sharing of germplasm through artificial breeding. The dominant breeds (e.g., Holstein-Friesian, Angus and Brahman) around the world are rapidly sharing elite sires. All of these elite animals carry known or as yet unrecognised mutations capable of causing congenital abnormalities. This inevitably leads to increased inbreeding, and more rigorous requirements of identification and management of inherited disorders is required, particularly those presenting before birth. There is recent evidence, particularly with the Angus breed of cattle, that the need for this approach is now increasingly understood. This chapter aims, with examples mainly from farm animals, to provide readers with an appreciation of and, potentially, the motivation to more systematically approach investigations of the disorders of development in a manner that can be of benefit to knowledge informing improved welfare of man and other animals.

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Abnormal Development Due to Genetic Disorders: Mutations and Chromosome Abnormalities

Mutations Causing Inherited Disorders

Inherited disorders arise from mutations involving errors in copying the DNA code, occurring at about $1x10^{-8}$ per base pair per generation in mammals (there are about $3x10^{9}$ base pairs in the mammalian genome). Only a small proportion of these mutations cause a sufficiently abnormal genotype to produce a significantly abnormal phenotype, although every individual animal and human is likely to be a carrier of multiple deleterious genes. Expression of a mutant gene depends on the influence it has on the synthesis and function of the protein encoded by the affected gene. Dominant inheritance occurs when only one copy of the abnormal DNA is required to produce the abnormal phenotype. Recessive inheritance requires two copies of the abnormal gene to produce the abnormal phenotype.

Dominant Mutations. In farm animals, a serious abnormal phenotype caused by a dominant mutation is usually recognised in the individual with the original mutation, enabling immediate recognition and removal of the mutation. This occurred with osteogenesis imperfecta (OMIA 000754), a collagen disorder described in Holstein–Friesian cattle, with recent confirmation of a mutation in COL1A gene (collagen type I alpha 1) (OMIA 002127). However, if the mutation during embryogenesis occurred after a sufficient proportion of embryonic cells had differentiated to enable subsequent normal tissue structure and function, so-called 'germ cell line mosaicism', the affected individual may have a relatively normal phenotype and the pattern of inheritance may initially appear to be recessive. However, as a significant proportion of germ cells carry the mutation, if the progenitor has many offspring, then the proportion of progeny affected can be useful in differentiating this pseudorecessive pattern of inheritance from true recessive inheritance. Furthermore, widespread dissemination of the mutation is unlikely because all progeny inheriting the dominant mutation will also have the abnormal phenotype, and the animal in which the mutation initially occurred is the only 'chimera' with a normal phenotype that can transmit the mutation. Thus dominant causal mutations are usually self-limiting, rarely achieving prevalence in the population of any significance.

Recessive Disorders. Mutations causing recessively transmitted disorders are far more significant in animal populations, particularly those in which widespread artificial breeding has occurred. The animal with a recessive causal mutation will transmit the mutation to half of its progeny, with the progeny that inherited the mutation then transmitting it to half their progeny. However, the abnormal phenotype will not occur at all until the successful mating of two descendants carrying the mutation. For Mendelian traits, 25% of the resulting progeny that inherit two copies of the mutation (one from each parent) will be homozygous and present with the disorder. An additional 50% of their progeny with normal phenotype will be heterozygous, carrying one copy of the mutation, and the other 25% will be genetically and phenotypically normal. If the recessive mutation is linked to a desirable trait, the prevalence of the mutant gene in the population will increase with selection, rising or declining as a result of changes in selection parameters and/or the structure of the population. The widespread use of an elite animal carrying the mutation is very likely to increase the prevalence of the mutation in the population, although the recognition of the abnormal phenotype as a heritable disorder is often delayed for many years and until after there are sufficient heterozygous matings to produce enough abnormal offspring for the disorder to be reported. This delay has enabled the spread of many mutations to continue until the disorder is spread globally and of economic significance, as occurred in Holstein–Friesian cattle, first with citrullinaemia (OMIA 000194), a defect in the urea cycle enzyme argininosuccinate synthase (ASS1), causing neonatal mortality due hyperammonaemia, and then with complex vertebral malformation (OMIA 001340), a defect in the solute carrier family 35 (UDP-N-acetylglucosamine transporter), leading to shortening of the spine and arthrogryposis.

When examining any animal with an unfamiliar disease presentation and particularly animals with congenital malformations, the clinician commonly considers the question, 'what genetic disorders occur in this age of animal and in this species and breed'. When faced with this situation, it is also important to remember that phenocopies of many disorders may exist with different genetic, toxic, or infectious causes. The deduction that a new disorder or syndrome is due to a genetic cause usually requires investigations to exclude toxic and infectious causes, often in circumstances in which a relatively simple pedigree analysis could have demonstrated heritability at a much earlier stage. Proper pedigree analysis to identify the closest common (maternal and paternal) ancestor is critical but is rarely well executed in practice. Accurate diagnosis of new genetic disorders is important for improving disease surveillance and breeding management, although it is also important to be careful not to suggest a genetic basis for a new disease entity without adequate evidence, as this can have serious reputational consequences for the producer and particularly the stud breeder.

The 'Founder Effect' and Artificial Breeding Risk

Due to the 'founder effect' in particular breeds, especially in cattle and dog breeds that have spread around the world from an initially limited numbers of animals and then more recently tended to be concentrated within a few families through the use of artificial breeding technologies, many recessively inherited defects are associated with both a particular breed and family within that breed. Each breed has a unique complement of recessively inherited defects (although similar defects can be observed in many breeds but are not necessarily all caused by the same mutation). Due to the restricted gene pools for many livestock breeds, we continue to see the emergence of new genetic disorders, often almost simultaneously in several countries sharing the same bovine gene pools. Public recognition of new defects is often delayed by a range of factors, including the genuine failure to diagnose the syndrome as a heritable disorder through to breeders choosing not to publicly disclose the presence of heritable disorders in their herds due to concerns of negative economic consequences as may have followed disclosure in the past. However, new developments in genetics allow for rapid development of tools to manage these disorders effectively. For the benefit of the breed, breeders, their organisations, and, importantly, their veterinarians should encourage early reporting and detailed investigation of suspected genetic disorders.

That newly recognised inherited disorders of cattle often present simultaneously on different continents is in part due to the efficient global distribution of the germplasm of elite individuals in the major breeds, combined with widespread use of artificial breeding. Some of the new disorders cause early embryonic death due to a lethal mutation and present as animals with reproductive failure. The early recognition of new and reemerging inherited diseases is important as it facilitates efficient control action by the various national cattle

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• **Fig. 9.6** Brachygnathia, cardiomegaly, and renal hypoplasia (BCRHS) syndrome in a dwarf poll Merino lamb.

industries to minimise the future negative economic and animal welfare influences of these diseases, particularly through heterozygote detection to enable avoidance of 'carrier–carrier' matings. In addition to development of heterozygote detection, investigations of inherited diseases should include detailed clinic–pathological documentation and pedigree analysis verification to ensure that issues such as phenotypic variation and misinformation in the use of molecular diagnostic tests for detection of heterozygotes are managed for the benefit of the breed society and industry.

Examples of Recently Emerging Inherited Disorders in Ruminants

Published reviews describing a number of the more widely recognised inherited developmental disorders include: cattle (Jolly & Windsor 2010), sheep (Jolly et al. 2004), and skeletal disorders of sheep (Dittmer et al. 2015) and congenital and postnatal disorders of the nervous system of cattle and sheep (Windsor et al. 2011a, b). Although fewer genetic developmental disorders of sheep have been reported compared with cattle, there are an increasing number of publications describing diseases in which genetic factors have been demonstrated or are considered most likely to be involved in their aetiology, including an increasing long list of suspected inherited neurological syndromes of mainly Merino sheep in Australia (Windsor et al. 2011b).

Small Ruminants

Some recognised developmental disorders in small ruminants suggested as inherited include: *congenital holoprosencepahaly* (OMIA 000478) in Border Leicester lambs; *spina bifida* (OMIA 000933) in Poll Wiltshire sheep in Australia and Dorper sheep in New Zealand; *fetal hydrops* (OMIA 000493) in Poll Dorset and Dorset sheep in Australia, in Merino lambs in South Africa, in Awassi lambs in Jordan, and in Cheviot and Cheviot-cross lambs with pulmonary hypoplasia in the United States; *hereditary chondrodysplasia* or 'spider syndrome' (OMIA 001703) in Suffolk sheep associated with a single base deletion in fibroblast growth factor receptor 3 (FGFR3) in the United States, Australia, and New Zealand; and *beta-mannosidosis* (OMIA 000626) in Anglo Nubian goats caused by a single base deletion in the lysosomal mannosidase beta A gene (MANBA).

Brachygnathia, Cardiomegaly, and Renal Hypoplasia Syndrome

Brachygnathia, cardiomegaly, and renal hypoplasia syndrome in Poll Merino sheep was recently described in Australia, characterised by dwarfism (Fig. 9.6) and multiple defects in several organs, including skeleton, heart, liver, and kidneys (OMIA 001595). The disorder was reported as 'brachygnathia, cardiomegaly, and renal hypoplasia syndrome' (BCRHS), and the disorder appeared to be transmitted as an autosomal trait with a recessive mode of inheritance (Shariflou et al. 2011). An annual incidence of the disorder in the discovery flock of up to 2.5% was recorded, and the mutation was mapped to the distal end of chromosome OAR2 (Shariflou et al. 2013).

Cattle

The review papers also include several now historical bovine disorders (Table 9.1), including *citrullinaemia* (OMIA 000194), *congenital myoclonus* (CM; OMIA 000689), and *maple syrup urine disease* (MSUD; OMIA 000627) of cattle, where identification of these disorders was important initially in Australia then globally. Clarification of CM and MSUD as the previously confused entities of so-called neuraxial oedema enabled more accurate diagnoses and eventually heterozygote detection tests for both diseases. Further, identification of CM as a congenital failure of spinal glycine receptor function (Gundlach et al. 1988) led to this disease providing an animal model for human hyperekplexia (Dennis et al. 2004). MSUD has also served as an animal model for the disease in infants (Harper et al. 1991a). The discovery of citrullinaemia may have provided the first example of an inherited congenital disease that became economically important due to widespread global use of a particular bloodline (the bull Linmack Kriss King) in the Holstein–Friesian breed. Citrullinaemia was also likely to have been the first cattle disease controlled by molecular heterozygote detection and also provided a useful animal model of the disease in infants (Harper et al. 1991b, Windsor & Agerholm 2009). Readers seeking further information on these disorders and the now vast array of developmental disorders that is being accumulated from many species are referred to the cited reviews and the numerous publications listed in OMIA. With many of the more recently emerging bovine disorders having mutations identified enabling heterozygote detection and with others yet to be published or

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• **Fig. 9.7** Polymelia in an Angus calf with an extra limb extending from the shoulders; likely to be developmental duplications defect, although image taken before discovery of the developmental duplications defect.

fully described, more detailed information is provided on some of these.

Developmental Duplication

Developmental duplication (DD; OMIA 002103) in Angus cattle emerged recently with initial recognition in Australia of neonatal calves with accessory limbs or polymelia in this breed (Fig. 9.7). This led to identification of a new mutation involving a single amino acid substitution in the NHLRC2 gene on Chromosome 26 (Table 9.1). Numerous pathological phenotypes have now been associated with the DD mutation that is transmitted with incomplete penetrance and variable expressivity. This includes DD homozygous animals present in the population as apparently normal individuals, although they may have subclinical lesions of DD (Denholm 2015). Sporadic cases of polymelia have been observed in other breeds, and these may involve the same or similar mutations, although it is possible that phenocopies of DD caused by other mechanisms may occur. DD causes losses from dystocia in calves with polymelia, costs of surgical amputations when performed, and the likelihood that significant embryonic mortality occurs, as suggested from genotyping information. A commercial molecular testing for the DD mutation is now available with these genotype nomenclatures adopted by the Angus breed societies:

- DDF Free: a noncarrier based on a DNA test result
- DDFU Free Untested: expected a noncarrier based on its recorded pedigree
- DDC Carrier: heterozygous for DD based on a DNA test result
- DDA Affected: homozygous for DD based on a DNA test result. Due to incomplete penetrance of the DD phenotype, a DDA animal may or may not express clinical signs of DD.
- DD__%: probability (%) that the animal is a DD carrier, calculated by 'GeneProb' software from the animal's BreedPlan recorded pedigree

DD appears to involve a fundamental neural tube defect, with both cranial and spinal dysraphism resulting from defective regulation of gene transcription in cell–cell signalling during transformation of the neural plate into the neural tube (neurulation). The wide range of abnormalities identified in DDA animals, either together in the same animal or in isolated phenotypes, has also been observed in dysraphic syndromes of other species. These include the following:

- *Polymelia:* Neonatal calves have one or more additional legs projecting from the body or head that originates close to the dorsal midline. This leg may appear relatively normal, have duplications of the digits including polydactyly or syndactyly, and the proximal bones may be vestigial, especially the scapula. The extra legs vary in size and dystocia is common.
- *Axial duplication:* Neonatal calves have an acephalic conjoined 'twin', with conjoined tissues having one or more projecting legs or resembling a mass attached to the posterior or flank of the host calf, with long bones usually within the tissue mass. Most present as dystocia and many die in the neonatal period.
- *Intradural teratomas:* A mature, well-differentiated, embryogenic teratoma is located within the dura of the CNS in the vicinity of the dorsal midline, usually in the occipital region or sacral area (where it may be associated with spina bifida).
- *Dermoid cysts and occipital skin flaps:* These may occur in similar locations to the teratomas and are small nodules of 0.5 to 1.5 cm in diameter, consisting of a fluid-filled cyst containing internally growing black hairs, with linkage to the midbrain or spinal cord. They are usually located within the dura, although may become extradural with subsequent development; some dermoids are located on the occipital bone at the base of a flap-like pouch of projecting skin.
- *Lipomas:* Some DDA cases have a fat tissue mass overlaying an embryogenic teratoma or dermoid cyst, sometimes located on the surface of the occipital bone near the midline and adjacent to an abnormal occipital foramen, although others may be intracranial.
- *Craniofacial dysmorphogenesis:* These are lesions of mild to severe facial defects, including maxillary brachygnathia and mandibular prognathia, cleft palate, defects of the muzzle, unilateral or bilateral micropthalmia, fusion of the eyelid or conjunctiva to the cornea, narrow palpebral fissures, hypoplasia of the oculomotor muscles, defects of the ocular lens, and hypoplasia of the optic foramen, sometimes with the retinal surface of the eye in proximity to the brain.
- *Craniofacial duplications:* Severe craniofacial dysmorphogenesis in DD results in diprosopus ('two faces') or possibly even dicephalon ('two heads').
- *Congenital arthrogryposis and hindlimb deformity:* Some DDA calves are born with deformed hindlegs, usually with arthrogryposis, but with no other signs of DD.
- *Postnatal ataxia:* Spinal cord tethering or compression may occur, although may not become clinically significant in DDA calves until the neonatal period or later, sometimes presenting with a postnatal onset of progressive hindlimb ataxia but no external signs of DD. Palpation of the spine may disclose a tumour along the spine or scoliosis associated with dysraphism and vertebral anomalies, although the cause of ataxia may only be detected by radiology or necropsy.
- *Embryonic deaths:* Dysraphic syndromes are associated with high rates of postimplantation embryonic death and resorption, with observations that much of the predicted mortality from genotyping information indicating mortalities occurred during embryogenesis.
- *Other presenting signs:* Other signs are expected in DD based on comparative pathological observations from human medicine and laboratory animals, and it is also expected that a proportion of congenital teratomas will become active later in life, with any expanding intracranial or intramedullary teratoma quickly becoming clinically significant. Dysgraphia has also been associated with thymic aplasia and aortic root anomalies.

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• **Fig. 9.8** Inherited arthrogryposis multiplex in a newborn Angus calf; note the similarities to arthrogryposis induced by Akabane virus (see Fig. 9.5).

Arthrogryposis Multiplex

Arthrogryposis multiplex (AM; OMIA 001465) or 'curly calf syndrome' (although this term is often also applied to AKAV-induced arthrogryposis in Australia) emerged in Angus cattle in 2008 in Australia and the United States, with affected calves displaying congenital contracture and fixation of multiple joints at birth and presenting at term, but dying shortly before or during parturition (Fig. 9.8). Hydramnion is very common in cows carrying these calves. Despite a markedly reduced birth weight (15–25 kg), dystocia is common due to the congenital arthrogryposis, with forelimbs fixed in flexion and hindlimbs usually fixed in extension; this assists the diagnosis. Lateral deviation of the facial bones and severe scoliosis and torticollis, with rotational deviation of the cervical, thoracic, and lumbar vertebral column and deformation of the ribcage and sternum, are common. Mild hydrocephalus has been observed in a small proportion of cases and palatoschisis has also been noted. The mutation identified an extensive deletion that encompassed the entirety of the ISG15 ubiquitin-like modifier (ISG15) gene, one or both of the 5' regulatory region of the hairy and enhancer split 4 (HES4) and of the agrin (AGRN) gene, enabling development of commercial heterozygote detection (Table 9.1).

Neuropathic Hydrocephalus

Neuropathic hydrocephalus (NH; OMIA 000487) is a heritable recessive disorder that was initially called 'water-head' because of the extreme cardiomegaly that occurs from severe hydrocephalus. Although NH segregates independently from AM in the same Angus bloodline, more AM affected calves were born than NH affected calves, suggesting there is considerable (50%–70%) embryonic or fetal loss in NH. Although both AM and NH cause dystocia, the severity of hydrocephalus and cranial enlargement in NH (Fig. 9.9) may require that prior incision of the domed cranium, drainage of the excess CSF, and manual removal of the cranial bones from the collapsed cephalic vesicle occur to enable traction-assisted delivery. The causative mutation responsible for this extreme form of hydrocephalus in Angus cattle has been identified but remains unpublished as of December 2017, although heterozygote testing is available.

Congenital Contractural Arachnodactyly

Congenital contractural arachnodactyly (CA; OMIA 001511) or 'fawn calf syndrome' (FCS) is a recessive heritable 'marfanoid' syndrome in newborn Angus calves that first emerged in 1998 in Australia and is now recognised in the United States and elsewhere.

• **Fig. 9.9** A prominent meningocoele in an Angus calf with neuropathic hydrocephalus, requiring resection of the head to enable delivery.

• **Fig. 9.10** Congenital contractural arachnodactyly in a neonatal Angus calf.

CA is manifest at birth as an abnormal posture characterised by elongated limbs, congenital contractures of the spine (kyphosis), and proximal joints of the hindlimbs, with prominent hyperlaxity of the distal limb joints at birth that largely resolves within a few days (Fig. 9.10). Almost complete resolution of the contractures occurs within a few months of birth provided the affected calf is ambulatory. Muscular hypoplasia is prominent in CA and persists into adulthood, although clinical severity is variable and detection of the arachnodactyly in CA may require observation of the affected calf in comparison to normal siblings. The mutation has been located and sequenced with the function of the affected protein involved in the endomysial connective tissue, and commercial heterozygote detection is available through the Angus breed societies (Table 9.1).

Proportionate Dwarfism

Proportionate dwarfism (OMIA 001485) has been reported in so-called 'miniature' Angus calves in Australia, although further investigations are required to determine whether these animals are the same genotype as those reported from two commercial NSW herds in the 1990s, with birth weights as low as 6.5 kg. Calves with proportionate body conformation but birth weights <15 kg are suspect, and although intrauterine growth retardation can occur from a number of mechanisms, at least some of these

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• **Fig. 9.11** A miniature Angus–Jersey crossbred neonatal calf and normal sibling affected with Angus proportionate dwarfism.

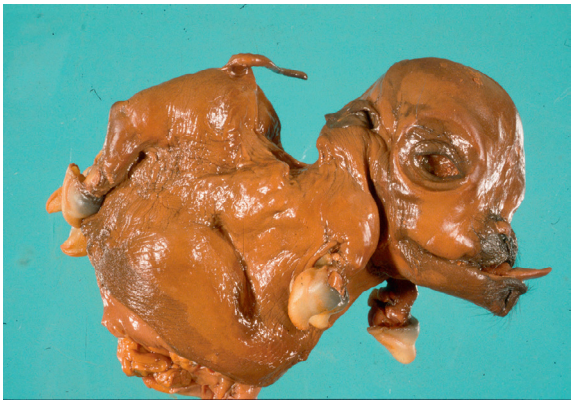
cases appear to be a form of primordial dwarfism, with affected calves being small for gestational age from conception. All these calves grow poorly (240 kg maximum), males are often cryptorchid, females are mostly infertile, and almost 30% of calves have some degree of scoliosis. Breeding experiments noted an average gestation length of only 8 months. The high frequency of occurrence of affected individuals (23/61 = 0.38 ± 0.06) among the progeny of matings of two clinically normal Angus sires to unrelated females of diverse breeding (Fig. 9.11) suggested the defect was transmitted by either an unstable mutant that changes at high frequency to the expressed dominant dwarfing allele during gametogenesis or a dominant allele with incomplete penetrance determined by an unlinked modifying locus (Latter et al. 2006). Other proportionate dwarfs in the Angus breed are reported to have normal birth weight but grow slowly after birth, typical of animals with reduced growth hormone function. With more than 200 forms of dwarfism reported in man, it is likely that many different forms of dwarfism are present in cattle, in addition to the proportionate and chondrodystrophic forms reported. Primordial dwarfism (associated with a higher than normal rate of failure of mitotic divisions and cell death in tissues throughout the body and consequent reduced life expectancy) will very likely be one of these.

Disproportionate Dwarfism

Disproportionate dwarfism (OMIA 001485) refers to one or more forms of dwarfism commonly and widely recognised in Angus cattle, in which mild chondrodysplasia is present, leading to aggressive culling practices in some herds for this suspected recessive genetic defect. Animals are typically smaller in stature than their cohorts with short legs and body, so the animal may appear to have a potbelly and a thick or blocky shape, and an elongated nose or shortened face (owner descriptions include a 'blocky conformation and a dished face with a long nose'). A recent molecular study sequenced four candidate genes and identified a nonsense R678X mutation in exon 15 of cGMP-dependant type II protein kinase (PRKG2) on Chromosome 6 that is involved in transcription of collagen 2 (COL2), consistent with the likelihood that this most common of phenotypes in Angus is a form of mild chondrodysplasia (Koltes et al. 2009).

Dexter Chondrodysplasia

Dexter chondrodysplasia (OMIA 001271) is a well-known defect in the Dexter breed in which, inadvertently, selection for a 'short legged phenotype' as the breed standard resulted in a high proportion of short-legged Dexters being heterozygous for the 'bulldog calf'



• **Fig. 9.12** Mummified Dexter fetus affected by chondrodysplasia, displaying the typical micromelia, shortened cranium, tongue protrusion, and shortened vertebral column and ribs.

• **Fig. 9.13** Chondrodysplasia in a Dexter fetus with micromelia, shortened cranium, tongue protrusion, and location of the abdominal viscera in a large umbilical hernia.

genotype. This is a nonviable fetus that may die *in utero* and be mummified (Fig. 9.12) or be aborted, although some calves survive until term, when fetal hydrops and hydramnios of the dam may also occur. Affected calves have severe chondrodysplasia manifest as micromelia, shortened cranium, palatoschisis, tongue protrusion, and shortened vertebral column and ribs (Fig. 9.13), resulting in multiple lobing of the lungs and eventration of the abdominal viscera through a large umbilical hernia (Harper et al. 1997). Two mutations on Chromosome 21 were identified in Australia involving two separate blood lines of Dexter cattle (Cavanagh et al. 2007), including a 4 bp insertion in exon 11 (2266_2267insGGCA) and

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a second, rarer transition in exon 1 (-198C>T) that cosegregates with the disorder (Tammen et al. 2007). In chondrocytes from cattle heterozygous for the insertion, mutant mRNA was subject to nonsense-mediated decay, showing only 7% of normal expression of aggrecan (ACAN), a protein enabling accumulation of fluid in cartilage matrix. These findings led to two PCR-based genotype tests that have helped to control the disorder worldwide.

Pulmonary Hypoplasia and Anasarca

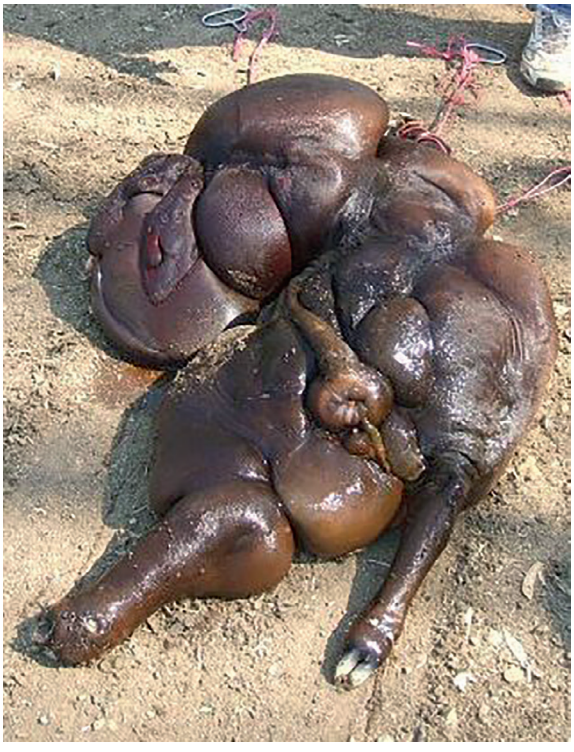
Pulmonary hypoplasia and anasarca (PHA) (OMIA 001562) was diagnosed in Dexter cattle in Australia when breeders reported the occurrence of severe fetal dystocia due to a huge 'bulldog-like' calf from cows that were known not to be heterozygous for Dexter chondrodysplasia. At necropsy, the calf morphology was characterised by hydrops fetalis (or anasarca) accompanied by pulmonary aplasia or severe hypoplasia, manifesting as an absence of lung tissue or presence of only rudimentary lungs in a large thoracic cavity (Windsor et al. 2006). Pedigree analysis indicated the defect is transmitted in an autosomal recessive manner, and the mutation has recently been identified but has not been published as of December 2017. PHA was also diagnosed in a single Brahman stud, with extreme anasarca and minimal pulmonary tissue present (unpublished). The extremely swollen fetus could only be delivered by Caesarean surgery (Fig. 9.14). Similar entities have been observed in Shorthorn and Maine–Anjou cattle in the United States, a Belted Galloway in Denmark, and Cika cattle.

Complex Vertebral Malformation Syndrome

Complex vertebral malformation syndrome (CVM; OMIA 001340) is a congenital lethal malformation in Holstein–Friesian cattle causing extensive fetal mortality, with only about 23% of affected fetuses surviving until Day 260 of gestation. Those that survive to term are mostly stillborn and display growth retardation with bilateral flexion of the carpal and metacarpophalangeal joints and rotation of the digits accompanying vertebral malformation, malformed ribs, and arthrogryposis of the tarsal and metatarsophalangeal joints (Fig. 9.15). Diagnosis of CVM is based on detection of a single base mutation in the gene SLC35A3, which results in insufficient function of a nucleotide–sugar transporter needed for normal development of vertebrae. Genotyping of almost 1494 Australian Holstein–Friesian bulls born between 1955 and 2001 revealed a carrier frequency of 12.79%, suggesting that numerous affected calves would have occurred in Australia but were likely to have been undetected or unreported.

Brachyspina

Brachyspina (BS; OMIA 000151) is a congenital lethal malformation of Holstein–Friesian calves, originally reported in Denmark in 2006, then Italy, the Netherlands, Canada and the United States. BS is characterised by reduction of the birth weight to about 10 kg despite a gestation period of normal or slightly prolonged length, obvious shortening of the spine, long slender limbs, inferior brachygnathism or micrognathism, renal and gonadal dysplasia (Fig. 9.16), and, in some cases, additional internal malformations (Agerholm et al. 2006). BS is transmitted in an autosomal recessive manner, and the mutation identified in the FANCI gene (Fanconi anaemia complementation group 1) has enabled a molecular test based on genetic markers to be developed for genotyping (Charlier et al. 2012).



• **Fig. 9.14** Pulmonary hypoplasia and anasarca (PHA) in a Brahman calf displaying extreme fetal hydrops with only a rudimentary proportion of pulmonary tissue found at necropsy.

• **Fig. 9.15** Complex vertebral malformation in a stillborn Holstein–Friesian calf with flexion of the forelimbs accompanying vertebral malformation, malformed ribs, and arthrogryposis.

• **Fig. 9.16** Brachyspina in a stillborn Holstein–Friesian calf with dwarfism, shortening of the spine, long slender limbs, and other malformations.

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Chromosomal Disorders

In Table 9.1 the chromosome on which the causative mutation(s) occurs is provided, reflecting the recent advances in molecular techniques that have enabled more rapid identification of the mechanisms that lead to inherited developmental disorders, including the disorders causing failures in gametogenesis and losses during blastogenesis, embryogenesis, or during fetal development, growth, parturition, and the early neonatal period. Although there are many thousands of genes, they are arranged in a limited number of chromosomes that can be visualised by karyotyping (Nicholas 2003). As some chromosomal abnormalities may have an important role in infertility in some species, where laboratory services are available, investigations of infertility should include cytogenetic examination of the karyotype to ensure major lesions of the chromosomes are absent.

Although chromosomes can be prepared from any dividing cell, peripheral blood lymphocytes collected in a heparinised blood sample is preferred, with short-term culture followed by addition of a spindle blocker to the medium, enabling the accumulation of cells at the mitotic metaphase for analysis of the chromosomes. Alternatively, chromosomes can be derived from fibroblast cultures established from skin subcutis samples. Simple staining enables chromosomes to be individually identified, the chromosomal number, morphology, and small abnormalities to be detected. Differential staining techniques identify either areas of highly repetitive DNA sequences (C banding) or bands of euchromatin and heterochromatin (G banding and R banding). Molecular genetic techniques, involving fluorescent in situ hybridisation (FISH), can also be used to identify specific DNA sequences or whole chromosomes. Chromosome spreads can be photographed, cut out, and arranged in an agreed order to construct a karyotype. Chromosome abnormalities may be numerical (e.g., aneuploidy or polyploidy) or structural, and may occur in the sex chromosomes (X or Y) or the nonsex chromosomes or autosomes. If an individual has more than one cell line it is referred to as mixaploid.

Numerical chromosome abnormalities include *aneuploidy* and *polyploidy. Aneuploidy* is when the chromosome number is almost diploid, but there are one or two chromosomes too many or too few, arising if there is nondisjunction during meiosis so that the chromosomes do not separate in a balanced fashion. X chromosome aneuploidy in females results in infertility because two X chromosomes are required for normal meiosis in the embryo, with a deviation resulting in oocyte atresia during embryonic development (i.e., XXX or triple-X syndrome, and XO or Turner's syndrome). An extra X chromosome in the male also results in infertility because the extra X chromosome interferes with spermatogenesis at puberty. Hence the phenotype is male but with small testes and azoospermia (i.e., XXY or Klinefelter's syndrome). Aneuploidy of the autosomes results in either too many (trisomy) or too few (monosomy) copies of a particular chromosome and its associated genes. The outcome of this depends on the genes involved, with either the individual displaying developmental abnormalities or, if these are not compatible with life, there is embryonic death. *Polyploidy* is when there are whole multiples of the haploid (i.e., half the diploid) chromosome number in excess and arises when there is a failure of the block to polyspermy or if there is retention of the first or second (or both) polar bodies during oogenesis.

Structural chromosomal abnormalities lead to outcomes that depend on whether genetic material has been lost (deletions) or simply rearranged (insertions, inversions, and translocations). When deletions have occurred, the developmental abnormalities depend on which genes have been lost and may be sufficiently serious to cause embryonic death. With rearrangements, balanced carriers of the anomaly are phenotypically normal, but problems may arise during meiotic prophase because the chromosomes have problems at pairing. This often results in nondisjunction and the production of chromosomally unbalanced gametes, producing unbalanced mostly unviable zygotes (trisomies or monosomies) if fertilisation occurs. Different abnormalities are found at different frequencies in the different species, with several of the more common anomalies in each species discussed.

Cattle

Centric Fusion Translocation

The most common chromosomal abnormality in cattle is a structural anomaly known as a centric fusion translocation in which two chromosomes fuse together near the centromere, resulting in a reduction in the chromosome number but little or no loss in genetic material. Over 30 different centric fusion translocations have been reported in cattle, of which the most common is the 1/29 translocation ('Robertsonian translocation'), which is found in a large number of breeds throughout the world. Heterozygosity (i.e., carrying one copy) for a centric fusion translocation results in a drop in fertility, the extent of which depends on which translocation is being carried. Animals heterozygous for the 1/29 translocation have a reduced fertility of the order of 5%. The drop in fertility is due to nondisjunction at meiosis, the production of chromosomally unbalanced gametes and embryos that undergo early embryonic death. The reduced fertility is manifested as a small increase in services per conception in the female and a lower nonreturn rate in the male. Carriers of some of the other centric fusion translocations have an even greater reduction in fertility, e.g., 46% with the 25/27 translocation in the Alpine Grey. Many countries will not allow the importation of animals carrying such translocations and artificial breeding agencies now often screen their bull populations and avoid using animals carrying translocations. Other less common chromosomal anomalies in cattle include phenotypically abnormal calves born with autosomal trisomies, cases of infertility in the bull due to the XXY Klinefelter's syndrome, and XXX infertile cows.

Freemartinism

Chromosomal analysis (see also Chapter 4) is also used in the diagnosis of *freemartins* because, in this condition, the fusion of the placental blood vessels not only allows Mullerian inhibition substance (MIS) and testosterone from the male to affect female development, but also allows the mixing of haemopoietic precursor cells, leading to the establishment of male and female cells in the blood of each twin. This can be detected by a simple chromosome analysis at any time after birth, enabling freemartinism to be predicted. No samples are required from the sire or dam. There is some debate as to the fertility of males born as a twin to a female. Although some surveys have shown reduced nonreturn rates, poor sperm freezability or poor sperm concentration and motility in the ejaculate of chimeric bulls, other surveys have shown no reduction in fertility and normal semen.

Sheep

Cases of infertility in the ram have been due to XXY Klinefelter's syndrome, with centric fusion translocations found in Romney, Perendale, and Drysdale sheep in New Zealand and in Romney Marsh sheep in the UK. However, unlike in cattle, centric fusion translocations have not been associated with a reduction in fertility

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in sheep. Reciprocal translocations have been reported in sheep with reduced fertility, but these are rare. In a survey of barren ewes, XX/XY (freemartinism) was the most common finding, and the XO (Turner's syndrome) has also been reported.

Goats

Centric fusion translocations have been found in Saanen and Toggenburg goats, although the effect on fertility is difficult to assess because of the confounding factor of the intersex gene associated with polledness in this species, nevertheless, it does seem that heterozygosity for a centric fusion translocation is associated with reduced fertility in the Saanen breed. The intersex gene is an autosomal recessive that is linked to the dominant gene for polledness, thus homozygous females for the polling gene are also homozygous for the intersex gene and are intersexes. Males that are homozygous for the polling gene are usually normal. However, 10% to 30% of these males may be infertile because of tubular blockage in the head of the epididymis.

Pigs

In the pig reciprocal translocations are the most common chromosome anomalies, with parts of chromosomes exchanged with little or no loss of genetic material. Carriers of reciprocal translocations are phenotypically normal but have problems at meiosis and produce unbalanced gametes, reducing litter size or even causing infertility. In Sweden 50% of boars culled for small litter sizes were found to be heterozygous for a reciprocal translocation, although most of these translocations appear to have arisen de novo and were not inherited, implying an environmental factor in induction of new translocations.

Horses

Chromosomal anomalies are an important cause of infertility in mares, with 50% to 60% of mares with gonadal dysgenesis having an abnormal chromosome complement. The most common chromosomal abnormality in mares is X chromosome aneuploidy (e.g., XO or XXX). Another common anomaly is XY sex reversal, with the animal presenting as a phenotypic female when it is in fact a genetic male. The incidence of such anomalies is uncertain, although in a study of 204 mares presented at stud for breeding, one mare was 65,XXX, one was 63,XO/64,XX, and one was a 64,XY sex-reversed male.

XO mares are usually small for their age, may have poor body conformation, usually fail to show oestrus, have small underdeveloped ovaries and a small 'infantile' uterus, and are infertile. XXX mares often present with the same clinical history as XO mares and, although some have shown irregular cyclic activity, all have been infertile. XY sex reversal is not uncommon in the horse, with most cases reported in Arabs and thoroughbreds. All are phenotypically female but are genetic males. The clinical signs are variable; some fail to show oestrus whereas others have irregular but strong oestrous behaviour. Some animals have had small, undifferentiated gonads, whereas others have had apparently normal ovaries with follicular activity. Most but not all are infertile. Mixaploid mares with normal as well as abnormal cells (e.g., XO/XX, XX/XXX, XO/XX/XXX or XO/XY) have been described, although diagnosis of the condition can be difficult. The fertility in mixaploid animals with a normal XX cell line is difficult to predict because, although most have been infertile, some have produced a foal. If the animal is actually showing oestrus and follicles are palpable on the ovary, it is probably advisable not to declare the animal infertile. Diagnosis of a chromosomal cause of a fertility problem cannot be made on the basis of the clinical history alone because animals with a normal karyotype present with the same history and clinical findings.

Few studies have been carried out on stallions with a fertility problem. Two colts have been reported with autosomal trisomies; one was a cryptorchid and the other had small testes. Structural abnormalities have also been found in Chromosome 1 in a stallion with a history of early embryonic death in mares that he covered.

Mules and Hinnies

The mule is a cross between a female horse and a male donkey, and the hinny is a cross between a female donkey and a male horse. The males of both crosses show abnormalities of chromosome pairing at the pachytene stage of meiotic prophase, and little or no mature spermatozoa are produced. Thus the males are infertile. Females are also affected during the fetal development of the germ cells, and most oogonia die as they are entering meiosis. However, sometimes a mature follicle is present in the adult, and, rarely, confirmed foalings have been reported in both mules and hinnies.

Dogs

Chromosomal abnormalities have not been associated with infertility in the dog except in one case of Klinefelter's syndrome and one case of prolonged prooestrus in a bitch with X chromosome monosomy (77,XO). Centric fusion translocations have been reported but there have been no consistent clinical findings. Their influence on fertility has not been investigated.

Cats

Chromosomal abnormalities are not common in the cat except in association with the tortoiseshell coat colour in the male. Many of these are XXY Klinefelter's syndrome cats, and they are always sterile. However, the mixaploid animals that have a normal XY male cell line may be fertile. Fertility presumably depends on whether the normal XY cell line is found in the testis. Fertile male tortoiseshell cats will breed as if they were normal XY toms and will transmit the coat colour genes by a Mendelian pattern of inheritance. Male tortoiseshell cats are considered uncommon, although in a survey of 9816 cats in the UK, of the 4598 males, 20 were male tortoiseshell cats. Because there is no reason to suppose that these chromosomal anomalies are only found in tortoiseshell cats, they are probably responsible for fertility failure in other cats but have gone undiagnosed because very few cats are examined cytogenetically. The XO condition has been reported and is therefore a possible cause of infertility in the female.

Abnormalities Due to Environmental Effects: Dietary and/or Toxic Disorders

Since the discovery of the teratogenic effects of hypovitaminosis A in pigs in 1933, the adverse influence of dietary factors on the development, growth, and survival of embryos have been regularly described. Teratogenic effects of vitamin, mineral, and trace element deficiencies and toxicities were demonstrated in experimental studies in rodents, and as these studies are informative for potential abnormalities in other species, they are briefly included here along with similar naturally occurring disorders of livestock populations. More detailed information is provided where there has been improved understanding in the teratogenic effects of deficiencies and toxicities.

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Undernutrition of both food (and water) is very likely to be important in adverse prenatal development, with increasing interest in the role of restricted protein and energy nutrition in induction of intrauterine growth retardation (IUGR) in livestock production (Long et al. 2009). IUGR adversely affects neonatal survival, postnatal growth performance, efficiency of feed utilisation, tissue composition (including protein, fat, and minerals), meat quality, long-term health of offspring, and adult onset of disease. IUGR involves genetic, epigenetic, and environmental factors including nutrition, as well as maternal maturity effect on the size and functional capacity of the placenta, placental vascular growth, utero–placental blood flows, transfer of nutrients from mother to fetus, the endocrine milieu, as well as embryonic development of myocytes, adipocytes, and other cell types. Growing evidence suggests that arginine-derived signalling molecules (nitric oxide and polyamines) play an important role in regulating these key physiological and biochemical processes. Potentially, modulating arginine-metabolic pathways that can enhance embryonic/fetal survival and growth may provide a useful approach to prevent and treat IUGR, although in many parts of the world, improving nutritional quality and quantity by improving feed resources for pregnant and lactating animals is a major challenge for improving livestock production efficiency. Maternal malnutrition was studied in mice deprived of food for limited periods before or during pregnancy, resulting in malformed offspring with the brain, ribs, and vertebrae affected.

Suspected nutritional chondrodysplasia of cattle refers to various syndromes of neonatal skeletal deformity reported in both free range and housed beef cattle in Europe, North America, South Africa, New Zealand, and Australia. Several descriptors have been used to identify affected calves including 'acorn calves, bulldog calves, congenital joint laxity, and dwarfism with congenital spinal stenosis' and, more recently, *congenital chondrodystrophy of unknown origin* (CCUO) associated with drought (White & Windsor 2012). Based on similarities of phenotypic appearance, affected calves display signs of disproportionate dwarfism (frequently with superior brachygnathia), shortened limbs (Fig. 9.17) and spinal deformities, with histopathology displaying disruption to normal endochondral ossification of long bones. Investigations including genetic, infectious, toxic, and nutritional aetiologies have been inconclusive with a common hypothesis promoted that the condition may result from a maternal nutritional deficiency. Recent investigations in Australia have shown the condition is associated with drought and with poor quality maternal nutrition (White & Windsor 2012). It is possible that CCUO calves result from multiple aberrant nutritional factors, with the epidemiological evidence suggesting manganese deficiency not strong, although the evidence for zinc deficiency appears stronger as a cause of CCUO. It was suggested that studies into short-term zinc deficiency in ruminants may prove helpful in future investigations.

• **Fig. 9.17** Weaner crossbred calf affected from birth with congenital chondrodystrophy of unknown origin (CCUO) associated with drought in southeastern Australia, displaying disproportionate dwarfism with shortened limbs from disruption of endochondral ossification.

Abnormalities From Deficiencies and Toxicities of Vitamins

Vitamin A

Vitamin A deficiency in the maternal diet causes ocular and facial malformations in pigs, commonly presenting as anophthalmia or microphthalmia, although malformations of the brain, the spinal cord, and other organs including the mouth and urogenital systems occurred, prevented by supplementation of the maternal diet by cod liver oil. In cattle, hypovitaminosis A during gestation causes abortion, resorption, stillbirth, or fetal malformations, with profound ocular changes and even blindness frequent in vitamin A-deficient calves. Primary vitamin A deficiency in growing cattle occurs most commonly from a lack of green feed during periods of grain-feeding in drought or in feedlots where carotene is excluded from the diet. Secondary deficiency may occur with a combination of chronic hepatic and intestinal disease as the liver is the principal storage site, and conversion of carotene to vitamin A occurs in the alimentary tract. Because vitamin A is required for osteoclast function and deficiency causes failure of bone remodelling, excessive bone is produced from where resorption should be occurring and may cause asynchrony between development of the CNS and bones in the skull, particularly in the lining of the optic canals through squamous metaplasia, reducing the space for the optic nerves.

Vitamin A toxicity during pregnancy can also produce malformations, with oral administration of 35,000 IU of vitamin A to pregnant rats producing various CNS abnormalities including exencephaly, with anophthalmia, microphthalmia, exophthalmos, cataracts, retinal defects, and open eyelids also described. Retinoic acid is the most teratogenic form of vitamin A and, in excess, can impair the normal development of many organ systems, inducing craniofacial and fetal limb abnormalities.

Vitamin B

Vitamin B complex deficiencies during pregnancy may adversely affect embryonic development and survival. The detrimental effect of chronic dietary deficiency of **thiamine** on embryonic growth and survival was established in rats and pigs, although no malformations were identified. Maternal **riboflavin** deficiency in pigs may result in anomalous limb development, with a deficiency in the diet of pregnant rats altering the development of the mandibles, long bones, and digits; thus a relatively mild degree of maternal riboflavin deficiency is considered teratogenic. **Niacin** (nicotinic acid) deficiency during pregnancy, produced by the incorporation of the antimetabolite, 6-aminonicotinamide, into the diet of rats, resulted in brain malformations, mainly exencephaly and hydrocephalus, although other brain malformations and ocular, urogenital, skeletal, and vascular abnormalities were induced by a single injection of this antimetabolite. Although **pyridoxine** (vitamin B6) deficiency during pregnancy may adversely affect perinatal development and survival, no definite evidence of teratogenicity has been noted, although the additional use of the antagonist (4-desoxypyridoxine) has been shown to be teratogenic to rat fetuses. **Folic acid** deficiency in pregnancy is also detrimental to

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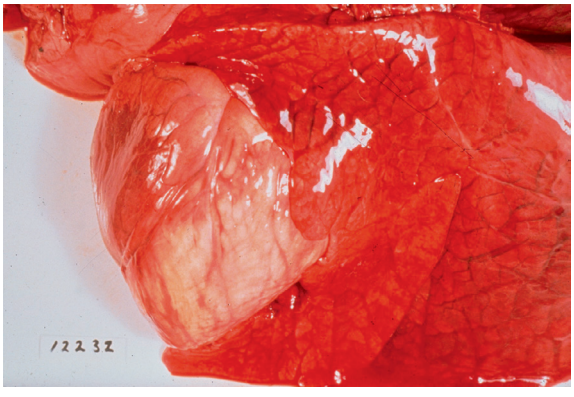
the development of rodent embryos, with teratogenicity increased by the addition of the folic acid antagonist pyrimethamine. Maternal folic acid supplementation in humans is associated with protection against deficits of neurulation; in rats, a deficiency of folic acid lasting only a few days during the early stages of embryogenesis produced a high rate of malformations, with hydrocephalus evident in the postnatal period and malformations of the brain, face, and mouth, as well as of the ocular, cardiovascular, urogenital, and skeletal systems observed. A diet insufficient in **pantothenic acid** fed to rats before and during pregnancy caused eye and brain abnormalities in the offspring, with exencephaly predominant. In pigs, reduced dietary levels of pantothenic acid resulted in fetal deaths and locomotor incoordination of the young. Maternal dietary deficiency of **vitamin B12** caused hydrocephalus in rat young, and even a marginal reduction in vitamin B12 intake of pregnant rats adversely influenced birth weight and postnatal growth.

Other Vitamins

Vitamin C has been shown to have no teratogenic affects in pregnant mice or rats, even at doses as high as 1000 mg/kg.

Vitamin D deficiency during pregnancy is harmful to the fetus, with skeletal abnormalities resulting from maternal rachitogenic diets occurring in rodents, pigs, and calves. Maternal **hypervitaminosis D** in mice and rats adversely altered osteogenesis of the long bones and ossification of the digits, with defects of the extremities increasing in degree of severity with advancing postnatal age.

Vitamin E deficient diets have also been shown to have a detrimental effect on maintenance of pregnancy, development, survival, and growth of rodent offspring, with severe maternal deficiency of vitamin E resulting in complete embryonic resorption. Supplementation with alpha-tocopherol enabled some embryo survival, with the incidence of malformations depending on the degree of vitamin E deficiency, including exencephaly, hydrocephalus, cleft palate, umbilical hernia, syndactyly, and tail deformities. Maternal vitamin E deficiency resulted in stillbirths in the dog, and neonatal deaths and incoordination of the hind legs in piglets, with grossly visible lesions of myodegeneration in congenital white muscle disease (WMD), including the heart (Fig. 9.18), observed in neonatal lambs, calves, and foals. Investigations of WMD should examine whether the issue is caused by vitamin E, selenium deficiency, or both, particularly as vitamin E and selenium supplementation reduces the incidence of these conditions. **Vitamin E excess** given orally at doses of 590 IU to mice during Days 7 to 11 of gestation produced neither embryotoxicity nor teratogenicity.



• **Fig. 9.18** Congenital cardiac white muscle disease in a neonatal calf due to vitamin E deficiency; note the lesions of extensive pallor of the myocardium due to myodegeneration.

Vitamin K deficient diets fed to pregnant rats caused cerebral haemorrhage in about one-third of the young, and abortions in pregnant rabbits. In cattle the ingestion of sweet clover hay or ensilage containing dicoumarol may result in death of newborn calves, especially when the dietary intake of dicoumarol exceeds 2 mg/kg body weight. Lambs are less susceptible than calves to the detrimental effect of dicoumarol ingestion by the mother.

Abnormalities From Mineral and Trace Element Deficiencies and Toxicities

Copper

Copper deficiency occurring during gestation in sheep may manifest as *congenital enzootic ataxia,* or 'swayback', and may be due to a primary deficiency due to low soil copper levels (as occurs on volcanic red soils after rain), or secondary to impaired gut absorption, usually from the presence of antagonists such as molybdenum. Copper is important for coenzyme for metabolic functions, and inadequate levels impede cellular respiration leading to energy deprivation. Affected lambs with swayback present from birth with motor dysfunction, including staggering, ataxia, recumbency, and occasionally blindness. They display bilaterally-symmetrical malacia (softening) of the cerebral white matter and defective myelinogenesis, leading to cerebral cavitation. A delayed form may also occur after several weeks of the postnatal period. In goat kids, copper deficiency is expressed mainly as the delayed form with lesions of cerebellar degeneration.

Copper toxicity is well described in grazing animals, particularly in Australia where exposure to pyrrolozidine alkaloid containing plants (particularly *Heliotropium europaeum, Echium plantagineum, Senecio* spp., *Amsinckia* spp., *Crotalaria* spp., and *Cynoglossus* spp.) is common and results in accumulation of hepatic copper until a precipitous release occurs, causing a hemolytic crisis and, often, mortality outbreaks. The potential teratogenic effects of these copper-accumulating situations is worthy of more detailed investigation.

Selenium

Selenium deficiency in neonatal lambs is typically manifest as ***congenital muscular dystrophy*** (white muscle disease/WMD) that can occur in newborn lambs, although is more commonly seen in lambs up to 3 months of age (subacute enzootic muscular dystrophy or 'stiff lamb' disease). Selenium is essential for a variety of roles, including: antioxidant actions in conjunction with vitamin E to prevent and repair cell damage; immune functions; and growth and fertility. In Australia, factors predisposing sheep to selenium deficiency include: acidic basalt/granite soils and sandy soils; annual rainfall over 450 mm; clover dominant pastures; and heavy or long-term fertiliser application, particularly with sulphur-fortified superphosphate or gypsum. Outbreaks of WMD and responses to selenium supplementation are more likely in lambs from ewes grazing in lush seasons on heavily fertilised, clover dominant pastures on granite soils. Similarly, weaner ill-thrift and scouring due to selenium deficiency will occur in 'the good years' but is less likely in dry years or under drought conditions. Selenium deficiency causing clinical WMD has also been observed in young lambs born to ewes that had been fed on wheat for most of their pregnancy, as some grains may be low in selenium, depending on the selenium availability in areas in which the crops were grown. As several disorders can cause similar clinical signs to WMD, clarification of the levels of both selenium (usually as plasma glutathione peroxidase) and vitamin E is advised, particularly as selenium therapy can be toxic if given excessively.

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Selenium toxicity is of potential concern where exposure to industrial contamination, grazing of seleniferous pastures, or where administration of excessive selenium supplementation to address deficiency concerns has been occurring (an increasing risk with promotion of selenium-fortified anthelmintic preparations). An overdose of selenium can be toxic, with an oral dose of 10 to 15 mg of selenium known to kill lambs, and an injection of 5 mg selenium potentially lethal to lambs. Selenium toxicity may be acute or chronic. Signs of selenium toxicity include: respiratory distress, blindness, staggering, head pressing, anorexia, salivation, abdominal pain, watery diarrhoea, convulsions, paralysis, and death. Administration of selenium (3 ppm) in the drinking water to mice during pregnancy caused an increase in the number of runts, with subcutaneous injection (40 µmol/kg of sodium selenite) to mice on Day 12 of pregnancy causing fetal mortalities and abortion. Interestingly, pretreatment with vitamin E (50 mg/kg) prevented abortion and maternal death but not fetal loss. Excessive selenium intake during gestation has been reported to have adverse effects on development and survival of the offspring in farm animals, with lambs born from ewes grazing on a seleniferous range displaying multiple malformations of the eyes (e.g., microphthalmia) and extremities (e.g., thickened joints, twisted and malformed phalanges), and hypoplasia of the reproductive organs, with almost 75% of 250 malformed lambs stillborn or dying shortly after birth. Hoof deformities occurred in calves and foals from dams receiving excessive amounts of selenium in their diet during gestation. Sows fed 10 ppm sodium selenite during gestation farrowed many dead piglets, with the survivors small and weak at birth.

Iodine

Iodine deficiency manifests as ***congenital goitre*** (CG), with clinical sign of hypothyroidism recognised when a neonatal lamb, kid, or calf has an enlarged thyroid gland at birth, increasing the risk of dystocia (Fig. 9.19). Neonatal goitre may be caused by an absolute deficiency of iodine when the pregnant females are grazing pastures grown on iodine deficient soils or be induced when ingested goitrogenic compounds in plants interfere with the uptake of iodine by the thyroid gland, causing iodine deficiency despite the females having normal plasma iodine levels. Hypothyroidism causes a reduction in metabolic rate, and the lambs and kids have reduced suckling behaviour and increased susceptibility to hypothermia, particularly in lambs with compromised development of wool follicles. Affected flocks and herds have reduced birth rates, increased stillbirths and neonatal mortality, with smaller birth weights and increased prevalence of 'wool break' within the flock (Robertson et al. 2008) and hypotrichosis in calves (Fig. 9.19). Goitrogens are found in *Brassica spp.* (canola, broccoli, cauliflower, Brussels sprouts, or cabbage), plus soy, pine nuts, millet, peanuts, and *Trifolium repens* (white clover). In *Brassica napus* (canola), the goitrogenic glucosinolates and S-methyl cysteine sulfoxide are released as isothiocyanates during digestion, interfering with the thyroidal uptake of inorganic iodine from the blood through the thyroid blood vessels, causing thyroid hypertrophy as a physiological response to the induced iodine deficiency.

• **Fig. 9.19** Marked enlargement of the thyroid due to colloid goitre in a stillborn crossbred calf with hypotrichosis; the producer is now supplementing with iodised salt.

Calcium deficiency is commonly occurs in adult cattle with postnatal hypocalcaemia (milk fever), causing flaccidity and paresis, reflecting the important role of calcium in controlling the release of the neurotransmitter acetylcholine that gates neuromuscular function. Hypocalcaemia syndromes also occur in feed-deprived sheep and in sheep consuming high-oxalate-containing pastures. However, as calcium is also critical for calcification and ossification processes, there is teratogenic potential of calcium deficiency when severe prolonged maternal deficiency during pregnancy adversely affects fetal skeletal development. Severe calcium deficiency of the mother may result in stillborn offspring or nonviable young in rats, cattle, and sheep; however, if the deficiency is mild or of short duration, fetal skeletal development remains unaffected due to mobilisation of maternal reserves from bone. **Calcium toxicity** from excessive calcium intake in the diet during pregnancy was shown to be detrimental to the developing fetus and young of pregnant rats, causing increased intrauterine mortality, reduced birth weights and postnatal growth rate, plus alopecia.

Magnesium deficiency is also common in grazing ruminants, particularly in lactating cattle sensitive to either low dietary magnesium or high potassium in lush green pasture, inhibiting rumenal magnesium absorption. Magnesium is important for neuromuscular function and deficiencies manifest as spasticity, convulsions, tremor, and tetanic spasms (grass tetany). Dietary magnesium deficiency during pregnancy may adversely affect survival and development of the fetus, with severe deficiencies in rats (0.2 mg magnesium/100 g diet) fed from Day 6 to 14 of pregnancy, resulting in very high (90%) rates of fetal resorption. When the deficient diet was fed only from Days 6 to 12, about 70% of the embryos survived, although malformations were common, including cleft lip, clubfoot, hydrocephalus, micrognathia, polydactyly, syndactyly, and heart, lung, and urogenital anomalies. An increase in the incidence of resorptions, malformations, and stillbirths was noted even with a relatively mild deficiency of magnesium (2.5–5 mg/100 g diet).

Iron deficiency in the diet before and during pregnancy causes anaemia in rat offspring with severe deficiency of iron resulting in very high neonatal mortality, with a few cases of ocular defects occurring in the offspring of rats deficient in iron during pregnancy. Inhibition of mitochondrial oxidative enzymes during fetal development is likely responsible for the high rate of fetal resorption and small fetal size observed in the rat in iron deficiency. Although this is not described in other species, the requirement for intensively housed neonatal pigs to receive iron supplementation in the immediate postnatal period is well known.

Manganese deficiency during pregnancy in rats and mice causes ataxia from absent or defective otoliths resulting in loss of equilibrium in the offspring, with severe shortening of the radius, ulna, tibia, fibula, and cranial bones. Supplementation with manganese prevented the neonatal ataxia, and studies suggested the possibility that aberrant development of the inner ear and the skeleton was

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caused by impaired synthesis of mucopolysaccharides. Ataxia of the young, as a consequence of maternal manganese deficiency, was also noted in pigs, and defects of the skeleton involving the limbs and vertebrae were attributed to maternal manganese deficiency in newborn rabbits and calves. Syndromes of drought-associated nonhereditary congenital chondrodysplasia in cattle in Australia (and elsewhere) have been commonly reported that likely involve environmental factors other than manganese deficiency, particularly as supplementation failed to ameliorate the disorder (White & Windsor 2012). **Excessive manganese** failed to produce teratogenicity in pregnant hamsters.

Phosphorus deficiency is common in many of the ancient soils of Australia, with grazing cattle often adversely affected, displaying signs of bone fragility and pica that increases the risk of botulism from consumption of decaying bones. As normal fetal growth and calcification of the skeleton depends on an adequate ratio of calcium to phosphorus in the diet of pregnant animals, studies of rats fed diets deficient in phosphorus noted in an increased occurrence of resorption of embryos; similarly, unmanaged phosphorus deficiency may potentially affect deleteriously the reproductive performance of grazing ruminants.

Zinc deficiency has deleterious effects on development and survival of embryos, with maternal dietary deficiency of zinc in rats fed an almost zinc-free diet (0.3–0.5 ppm of zinc) resulting in embryonic death or malformations, with 40% to 50% of the implantation sites resorbed and nearly all survivors displaying malformations affecting almost every organ system. Malformations included anencephaly, hydrocephalus, exencephaly, and, less commonly, defects of the eye, lip, palate, heart, lung, spine, urogenital organs, and pituitary and pineal glands. Zinc deficiency in early pregnancy in rats causes abnormal cleavage and blastulation in preimplantation embryos, suggesting the malformations result from impaired nucleic acid synthesis. Zinc deficiency in sows during the last trimester of pregnancy resulted in a moderate degree of hydrocephalus in some piglets and irregular ossification centres and hypoplasia in the developing skeleton, with excessive umbilical haemorrhage causing reduced neonatal survival. Excessive amounts of zinc in otherwise adequate diets seem to have little adverse effect on embryonic development and survival in hamsters and rats.

Abnormalities From Industrial Pollutant and Agricultural Chemical Toxicities

With exception of a limited number of pharmaceutical agents that are of large molecular size, or biotransformed into inactive metabolites, or firmly bound to maternal tissue, most chemicals used in industry and agriculture readily cross the placental barrier and enter the embryonic milieu where they may interfere with the various processes of prenatal development and growth, including embryonic death, intrauterine growth retardation, and malformation (Szabo 1989). These effects on prenatal development may depend on the specificity and embryotoxic or teratogenic potentials of the agent, on the time of exposure during pregnancy, and on the susceptibility of the species. A few of the various classes of chemicals, drugs, and pharmaceutical agents that have been found to cause teratogenic effects in domestic animals are discussed in this section. As the literature is vast on the many drugs that are known to adversely influence prenatal development in experimental animals if given at sufficient dosage by the appropriate route to a susceptible animal species at the sensitive stage of embryogenesis, the reader is advised to seek more detailed information from other sources (McGeady et al. 2017).

Atrazine is a synthetic triazine insecticide, herbicide, and stabilising compound used in plastics. It is capable of causing altered gene expression and disruption of endocrine function even at low concentrations. It is of particular concern as a pollutant of wildlife populations as it causes abnormities of the gonads in fogs and male rats. Atrazine induces production of aromatase that converts testosterone to oestradiol, adversely affecting male fish and amphibians with immunosuppression increasing the susceptibility to infectious agents in aquatic environments.

Bisphenol A is also a stabilising compound used in plastics but can leach into water at levels that can affect gonadal development in rodents through oestrogenic activity. In addition, *in utero* exposure in rodents induces cellular changes that lead to tumour formation on exposure to oestrogens or carcinogens later in life. There are concerns that accumulation of bispenhol A in children from widespread use of such compounds and their persistence in the environment may create future toxicity problems in populations of wildlife, domestic animals, and humans.

Cadmium toxicity is of potential concern in grazing animals and is a known teratogen in rodents with intravenous injection of 2 mg/kg cadmium on Day 8 of pregnancy producing severe facial deformities, including mandibular defects and cleft palate in hamsters. Combined treatment with cadmium and lead resulted in synergistic effects with malformations differing from those produced by either of these two agents given alone. Intraperitoneal injection of cadmium sulphate on Day 7 of pregnancy in mice caused fetal exencephaly, with cellular changes in the embryonic nervous tissues detected relatively soon after cadmium injection and haemorrhages a few days later.

Dioxin is a halogenated hydrocarbon used widely as an herbicide, linked to congenital anomalies in human populations when used as a defoliant in the Vietnam War. Exposure of pregnant mice to dioxin induces defects of the palate (cleft), brain, kidney, and other organs, with teratogenic effects suggested due to interference with epidermal growth factor or transforming growth factor.

Lead toxicity occurs commonly in grazing ruminants, particularly cattle exposed to lead in paint or batteries. Moreover, high levels of lead in drinking water, vegetables, and air, are considered as risk factors for toxicity in children. However, the teratogenic effects of lead toxicity in ruminants is not well known, although it is of potential concern as embryotoxic and teratogenic effects have been demonstrated with lead exposure in mice, rats, and hamsters causing skeletal, vertebral, and urorectal defects. The teratogenic effect of lead in the mouse was markedly enhanced when the pregnant animals were kept on a diet low in calcium. Lead accumulates in fetal tissues and, as in children born to mothers exposed to subclinical levels of lead had behavioural disturbances, lead pollution in humans and other animals is of serious concern in a number of environments.

Mercury as methyl mercury ingested during pregnancy causes damage to the fetal CNS of humans, with cerebral palsy, blindness, mental retardation associated with lesions of microcephaly, and cerebral atrophy.

Organochlorines, or chlorinated hydrocarbons, are environmentally polluting chemicals including DDT (dichlorodiphenyltrichlorethane), Heptachlor, and Dieldrin, used widely as pesticides until they were banned in the mid-1970s. These compounds were notorious for accumulating in the soil on farms and in the fat of predated animals, leading to a concern of pollution of the human food chain, and a precipitous decline in the populations of predatory birds, especially condors, eagles, and falcons, due to egg shell fragility. The metabolic product of DDT is DDE, reported to mimic

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oestrogenic activity or inhibit androgen effectiveness. Feminisation of fish populations, declining human sperm counts, and increased frequency of breast cancer are commonly attributed to DDE. DDT fed to animals can cause toxicity of the embryo and fetus.

Polychlorinated biphenyls (PCBs) are halogenated aromatic hydrocarbons used widely for industrial purposes until they were banned in the 1970s due to toxicity and pollution concerns. PCBs are teratogens, carcinogens, neurotoxic, and immunosuppressive, with indications they caused decline in reproduction of otters, seals, mink, and fish. High doses in pregnant women caused reduced fetal growth rates and skull abnormalities, plus deformed nails and hyperpigmentation of gums and other tissues, with deficient cochlear development and deafness in rats. As some PCBs structurally resemble oestrogens and thyroid hormone, they likely exert teratogenic effects through disruption of endocrine functions.

Abnormalities From Veterinary Therapeutic Toxicities

Azoles (e.g., fluconazole) are used for fungal therapy but contraindicated in pregnancy as they have been shown as teratogenic in rodents and caused skeletal and cardiac anomalies in babies born to mothers administered high doses in gestation.

Angiotension–tension converting enzyme inhibitors administered in mid- to late gestation to pregnant humans have caused pulmonary hypoplasia, IUGR, and skull and renal abnormalities, with fetal and neonatal mortalities from severe fetal hypotension.

Benzodiazepines (e.g., diazepam) are commonly used as sedatives in humans and occasionally animals, but readily cross the placental barrier where, in the first trimester, they may cause fetal craniofacial abnormalities in babies.

Benzimidazoles are widely used as anthelmintics in livestock production, and some earlier preparations (e.g., parbendazole, cambedazole) produced embryonic abnormalities, including skeletal, renal, and vascular anomalies, when administered to pregnant ewes between Days 14 and 24 of gestation.

Doxycycline is used commonly in companion animals and caution is advised as it presents embryotoxic risks when administered to humans in the first trimester of pregnancy.

Griseofulvin is commonly used for oral antifungal therapy in companion animals, although there are risks of teratogenicity in pregnant cats and horses.

Methallibure has been used to control oestrus in sows but causes cranial and limb deformities when administered in early gestation.

Streptomycin has long been associated with deafness in babies from deleterious effects on cranial nerve VIII.

Abnormalities From Plant and Fungal Toxicities

Dysphania glomerulifera is an Australian native chenopod that is the likely cause of outbreaks of *congenital biliary atresia* (BA) in neonatal lambs (and two calves) born to mothers restricted to grazing the plant during gestation after droughts in Australia in 1964, 1988, 2007, and 2013. BA is a rapidly progressive and destructive fibrotic disorder of undetermined aetiology, affecting the extrahepatic biliary tree of neonates in many species but particularly in humans, being the most important cause of infantile liver transplantation. The Australian outbreaks affected between 14% and 100% of neonatal lambs born to ewes grazing the banks of two large water reservoirs during gestation in which abundant *Dysphania sp.* had been growing when water levels were low. Affected lambs displayed white faeces and failed to thrive, with necropsy confirming the dysgenesis of the gall bladder (see Fig. 9.1) and microscopic lesions suggesting a toxic hepatopathy associated with choledysgenesis (Harper et al. 1990). The ewes and other adult animals were unaffected. Recently, a new isoflavonoid compound 'biliatresone' was isolated from *Dysphania sp.* recovered from the vicinity of an outbreak of BA in sheep, causing selective destruction of the extrahepatic biliary system of larval zebra fish (Koo et al. 2016). Further, as a mutation that enhanced biliatresone toxicity was mapped to a region of the zebra fish genome that has conserved synteny with an established human BA susceptibility locus and the toxin also caused lesions in neonatal mouse extrahepatic cholangiocytes in cell culture, it was proposed that congenital BA could be due to exposure to an environmental toxin during gestation (Lorent et al. 2015).

Veratrum californicum (western false hellebore or skunk cabbage) consumed by pregnant ewes early in gestation can produce craniofacial and skeletal defects, including cyclops, in up to 25% of lambs born. Studies identified that embryonic death occurred in ewes exposed at high doses on Day 14, whereas the facial and limb deformities occurred in lambs from ewes exposed to less toxic levels. When pregnant ewes were gavaged with root–rhisome material for 3 days near Day 29 of gestation, lesions of shortened fetal metacarpal, metatarsal, and tibial bones were identified in the lambs, and some displayed arthrogryposis (Keeler & Stuart 1987). These findings confirmed that the severity of defects varied with level of toxicity and gestational stage, and identification of the toxins cyclopamine and veratramine enabled studies on craniofacial defects in humans and other species.

Theories explaining the pathogenesis of craniofacial malformations have included: a defect in the prechordal mesoderm; failure of the prosencephalic organising centre to induce development of the anterior part of the neural tube; abnormal position of the notochord; a defect in the neural plate or the neural tube; and an abnormality in the hyaluronate-rich extracellular matrix through which the neural crest cells migrate. However, more recent discoveries of the role of the soluble morphogen Sonic hedgehog *(Shh),* a vertebrate orthologue of Drosophila hedgehog, as a key signalling factor in the regulation of craniofacial skeleton development in vertebrates, may offer more fundamental understanding of how CFDs occur. First identified in 1993, *Shh* now has multiple roles in development and disease (Dwokin et al. 2016). The *Shh* gene is expressed in the notochord, the floor plate of the neural tube, the posterior limb buds, and the developing gut. However, mutations in *Shh* classically lead to defects in midline structures, particularly the face and eyes and brain, such as holoprosencephaly, plus numerous defects of nonneural tube origin, including ocular hypertelorism, midface hypoplasia, and cleft lip only or cleft lip/palate defects. *Shh* mutations also cause defects of the eye, including coloboma (missing eye tissue) and micropthalmia (abnormally small eyes). As *Shh*-mediated craniofacial defects are largely caused by a loss of neural crest cell fidelity and survival within the pharyngeal arches, a holistic role for *Shh* genes in craniofacial development is likely, and mutations in *Shh* may yet prove to be also clinically relevant in the aetiology of CFDs in animals, particularly sheep.

Lupinus spp. caused bovine skeletal deformities and cleft palate in the United States in the late 1950s when newborn calves affected by 'crooked calf disease' (arthrogryposis) were attributed to maternal ingestion of *Lupinus* spp. Then in the late 1960s and early 1970s in the United States, epidemics of skeletal malformations in pigs were recorded, associated with ingestion of poison hemlock (*Conium maculatum*) and burley tobacco (*Nicotiana tabacum*). The malformations in newborn pigs appeared similar to those in

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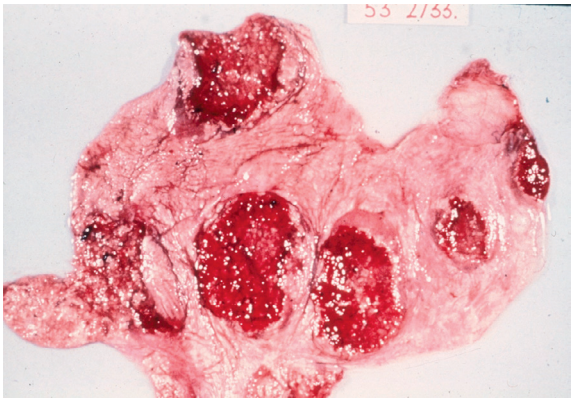
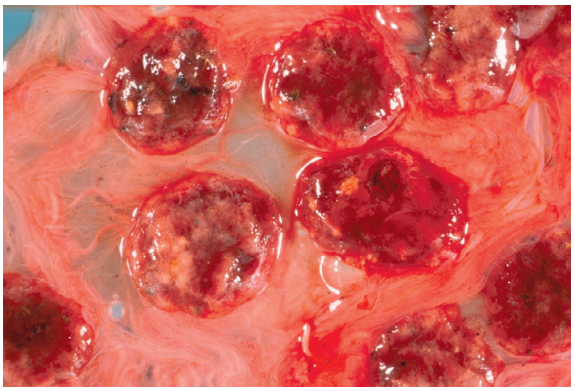
crooked calf disease, with the proposed mechanism of action for *Lupinus*-induced malformations and cleft palate attributed to reduction in fetal movement, with the teratogenic effects of *Lupinus, Conium,* and *Nicotiana* spp. believed to be identical (Welch et al. 2012). Ultrasonography demonstrated a direct relationship between reduced fetal activity and severity of contracture-induced skeletal defects and cleft palate in sheep and goats. *In utero* positioning and/or abnormal tendon and ligament development resulting from lack of movement and the cleft palate is believed to result from mechanical interference by the tongue between the palatal shelves at the programmed time of closure (Panter et al. 1990). A goat model was developed for the study of cleft palate in humans, with research focused on the privileged period of fetal scarless healing after *in utero* surgery to repair cleft palates early in gestation.

Mycotoxins are metabolites produced by fungi (e.g., aflatoxin from *Aspergillus flavus*) and, when ingested, often through spoiled feed, have produced teratogenic, carcinogenic, mutagenic, and immunosuppressive effects in farm animals. *Aspergillus fumigatus* and *Mortiarella wolfii* are fungi that are capable of causing sporadic abortions from infections of the bovine placenta and fetus in late gestation, with lesions often grossly visible on the epidermis of aborted calves (see also Chapter 26).

Other toxic plants are very likely to cause developmental abnormalities as there are many plant species throughout the world that containing toxins that are structurally similar to the alkaloids known to be teratogenic. Although these plants may appear in the diets of humans and other animals, toxicity and teratogenicity are a matter of dose, rate of ingestion, time of insult, and alkaloid concentration and composition in the plant; thus toxicity/teratogenicity may not always be readily correlated with ingestion. For example, there is circumstantial evidence that the alkaloids in *Lathyrus latifolius* may have been the cause of arm and hand deformities in a baby boy, via maternal ingestion of goat's milk (Welch et al. 2012).



• **Fig. 9.20** Diffuse severe necrotising placentitis accompanying fetal infection in cattle due to infection with *Brucella abortus.*



• **Fig. 9.21** Multifocal moderate hypertrophic placentitis with enlarged cotyledons of an ovine placenta accompanying fetal infection in sheep due to infection with *Toxoplasma gondii.*

Abnormal Development Due to Infections

Congenital malformations of animals caused by viral infections with pathogenicity for developing organs of the fetus (see also Chapter 26) are extremely important in livestock production in many parts of the world, and there is recent renewed interest in these infections because of the occurrence of Zika virus in humans (Kim & Shresta 2016). Outcomes of teratogenic virus infections reflect the often complex interactions between the host–pathogen–environment (HPE) factors including virulence and transmission potential of the infecting viral strain, host factors including species, breed, age, and immune status, plus, in arthropod-borne infections, vector survival and spread as determined by dynamic environmental influences including climate change. Further, the pathogenesis of the lesions produced by teratogenic viruses reflects the gestational stage of the fetus when infected. Typically, the cells of the CNS and sometimes other organs become susceptible to tissue damage after the onset of early immunocompetence (usually after 80 days of gestation), indicating that the disease is a consequence of cellular damage from both viral and inflammatory mechanisms. After development of more complete immunocompetence (usually after 160 days of gestation), these viral infections are usually cleared adequately by immune mechanisms and infected animals are largely spared from congenital malformations.

Intrauterine infections by bacterial and protozoan parasites are also common in ruminants, with the invasion of the placenta and fetal tissues causing abortion, stillbirth, and neonatal disorders, although the extreme deformities as caused by the teratogenic viruses are rarely observed by these pathogens. Bacterial infections that cross the placenta, causing placentitis (Fig. 9.20) and fetal infection that cause abortions and neonatal mortalities, include *Listeria* spp., *Brucella* spp., *Leptospira* spp., *Chlamydiophila* spp., and others and are discussed elsewhere in this book. Similarly, the common Apicomplexan protozoal infections *Neospora caninum, Toxoplasma gondii,* and several of the *Sarcocystis* spp., with sexual reproductive life cycles in the alimentary tract of carnivores, regularly invade in tachyzoite form the nervous systems, and other organs of ruminants during gestation. Congenital infection of both clinically diseased and normal calves by *N. caninum* is well described (Kessell et al. 2011), with neosporosis mainly a disease of cattle (Boulton et al. 1995, Atkinson et al. 2000), although sheep and goats have been infected (Bishop et al. 2010). The primary host for *N. caninum* is the dog, including dingoes in Australia (King et al. 2010), whereas the primary host for *T. gondii* is the cat. Toxoplasmosis commonly infects the fetus in many species, with congenital infection recorded in dogs (Al-Qassab et al. 2009), and severe lesions in the CNS have been observed in aborted lambs, with hypertrophic lesions observed in the placentomes (Fig. 9.21). Cattle appear rarely infected. The protozoan *Babesia argentina* infection in parasitemic cattle has also been observed to cause congenital infections. As the nonviral infections of ruminants only occasionally produce congenital deformities, usually involving the neuromuscular system as a consequence of CNS infection compromising lower motor neuron function, readers are referred to further discussion elsewhere in this book or recent reviews of the subject (Kessell et al. 2011).

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Cattle and Small Ruminants

Bovine Viral Diarrhoea Virus

Bovine Viral Diarrhoea virus (BVDV), in the genus *Pestivirus* of family *Flaviviridae,* exists as two genotypes, BVDV-1 and BVDV-2, with each genotype containing the two biotypes characterised by their capacity to affect the cells in laboratory cultures, i.e., cytopathogenic (cp) and noncytopathogenic (ncp) biotypes (Agerholm et al. 2015). Although both BVDV genotypes are likely to be distributed globally and cause similar disease manifestations, BVDV-1 strains occur more commonly, and BVDV-2 strains are considered more virulent. BVDV infection with the ncp biotype of either genotype 1 or 2, occurring before and during gestation, may result in a variety of clinical–pathological outcomes, depending on the immunity of the dam, gestational stage, fetal immunocompetence, virus biotype, and strain virulence. Fetal infection with ncp BVDV before 79 days of gestation usually results in embryonic death or persistence infection due to immune incompetence, although infection up to 6 months of gestation may result in mummification, abortion, premature birth of a live, weak, undersized or apparently normal calf, and various congenital malformations. Fetal infections with ncp BVDV between Days 79 and 150 of gestation, when the CNS is most vulnerable to cellular damage and fetal inflammation, may cause lesions of cavitation of the cerebrum (i.e., hydranencephaly and porencephaly) and, more commonly, the cerebellum, causing various degrees of dysplasia, most commonly cerebellar hypoplasia of varying degrees (see Fig. 9.4). Congenital malformations of BVDV may also include ocular lesions (i.e., micropthalmia) and alterations of thymus (i.e., thymic hypoplasia), bones (i.e., brachygnathia inferior, growth retardation), hair coat (i.e., hyotrichosis or alopecia), lung (pulmonary hypoplasia), kidneys (i.e., renal hypoplasia), and changes of the hair coat (hypotrichosis/alopecia, curly hair). As BVDV infections causing lesions resulting in malformations usually follow commencement of immunocompetence, affected calves will generally be seropositive. A recent review of the lesions in BVDV-induced congenital malformations includes detailed description of the CNS lesions (Agerholm et al. 2015).

Akabane and Aino Viruses

Akabane and Aino viruses are closely related arboviruses of the genus *Orthobunyavirus,* within the Simbu serogroup in the family *Bunyaviridae.* Akabane virus (AKAV) was first suspected of causing the congenital bovine abnormalities of hydranencephaly and arthrogryposis in 1974 in Japan and a few years later in Australia. As outbreaks of AKAV have also sometimes been accompanied by other abnormalities, including abortion, stillbirth, polioencephalomyelitis, and possibly microencephaly, it is generally considered the most pathogenical of the Simbu serogroup viruses. AKAV is readily transmitted to cattle by the insect vector, the biting midge *Culicoides brevitarsis* in tropical and subtropical 'endemic' regions of Australia, with outbreaks or sporadic cases of hydranencephaly (see Fig. 9.2) and arthrogryposis (see Fig. 9.5) reflecting the distribution of the vector to areas where naïve cattle are located or movement of naïve pregnant animals into the endemic zone. In these areas AKAV regularly infects young female cattle before gestation, enabling immune protection of the fetus in subsequent pregnancies. Clinical signs are rarely observed in these endemic zone above the so-called 'brevitarsis line', although movement of naïve pregnant cattle into the endemic zone during periods when the fetal CNS is vulnerable to damage from the virus and inflammation has caused extreme financial losses and welfare concerns (Jago et al. 1993). In cattle, infection between Days 79 and 104 of gestation results in hydranencephaly due to cavitation of cerebral tissue, whereas exposure at Days 103 to 174 causes arthrogryposis, resulting from lesions of dysplasia and loss of lower motor neurons in the spinal cord, occasionally accompanied by focal Wallerian-type degeneration in the CNS. Because arthrogryposis occurs later in gestation, it is usually observed before the occurrence of hydranencephaly in herds with a relatively prolonged parturition period. Clinical signs of AKAV infection are not usually observed during the postnatal viraemic period in cattle in Australia, although encephalomyelitis has been suspected and has occasionally been reported in calves and adult cattle infected postnatally in Japan. Placentitis is not a feature of transplacental transmission of AKAV, with calves affected by hydranencephaly frequently born alive with behavioural abnormalities, described as 'dummy calves'. Lesions in the brain produce cerebral defects, from mild porencephaly to severe hydranencephaly with only a small rim of cortical tissue remaining, although hydrocephalus and, in rare cases, mild cerebellar hypoplasia have been observed. Calves with arthrogryposis frequently cause dystocia. As AKAV infections cause lesions after commencement of immunocompetence, affected calves will be seropositive. Although an efficacious, commercially available vaccine was developed for use in Australia in the 1980s, it was withdrawn after a short period due to market failure. A recent review of the lesions in AKAV-induced congenital malformations includes detailed description of the CNS lesions (Agerholm et al. 2015).

Schmallenberg Virus

Schmallenberg virus (SBV) is also a member of the genus *Orthobunyavirus* within the Simbu serogroup of the family *Bunyaviridae* and was identified in 2011 as the first pathogenical orthobunyavirus of ruminants to be recognised in Europe. As with other orthobunyaviruses, SBV is transmitted by biting midges, the *Culicoides* spp. Although the origin of SBV remains undetermined, it was first detected in the vicinity of the German rural town of Schmallenberg near the Dutch border, and then identified in 27 European countries within 2 years, indicating efficient transmission by the insect vector. Infection of naïve cattle during the vulnerable period of the fetal CNS between gestational days 60 and 180 results in lesions of the CNS producing syndromes of congenital hydranencephaly or arthrogryposis. The severity of lesions in the brain and spinal cord reflects an interaction between the developing fetal CNS tissue and developing immunocompetence, plus the virulence of the SBV strain. As with AKAV, infection during early gestation results in moderate to severe dysplasia of the brain and spinal cord, with infections in later gestation resulting in encephalomyelitis. Placentitis is not a feature of transplacental transmission of SBV, although unlike in AKAV in which calves with hydranencephaly are frequently born alive, most SBV-malformed calves are stillborn with a significantly lower body mass than unaffected calves. Lesions in the brain produce cerebral defects, ranging from microencephaly or porencephaly in combination with hydrocephalus and lissencephaly to severe hydranencephaly with only a small rim of cortical tissue remaining, although cerebellar hypoplasia may also be observed. Lesions of the spinal cord cause varying degrees of athrogryposis and are often associated with scoliosis and/or kyphosis of the thoracic part of the vertebral column. As SBV infection causes lesions after commencement of immunocompetence, affected calves will be seropositive. A recent review of the lesions in SBV-induced congenital malformations includes detailed description of the CNS lesions (Agerholm et al. 2015).

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Blue Tongue Virus

Blue tongue virus (BTV), the cause of blue tongue (BT) disease in mainly small ruminants, is a member of the genus *Orbivirus* within the *Reoviridae* family; currently, 26 serotypes are distinguished, most commonly occurring in tropical and subtropical areas. A large scale epizootic of BTV infection, causing high morbidity and mortality on a number of the Mediterranean islands and later in most countries bordering the northern Mediterranean coast, occurred between 1999 and 2002, with over 0.5 million sheep affected in Italy and Sicily alone. BT disease is mostly confined to sheep, although some highly virulent strains occasionally cause disease in goats; there was no evidence of disease in cattle until the emergence of an unusual strain of BTV serotype 8 (BTV-8) in The Netherlands and Germany in 2006, with subsequent spread through most of Western Europe and into England and southern parts of the Scandinavian countries. BTV-8 can cross the placenta in ruminants causing congenital defects, is excreted in semen, and can cause mild disease in a very small proportion of infected cattle. However, cattle are generally considered to be the amplifying mammalian host and can carry infectious virus for many weeks, long after the development of an antibody response.

The clinical outcome of a BTV infection, as with other arthropod-borne viral entities, is dependent on numerous HPE interactions. Seasonal outbreaks of BT due to BTV serotypes 1, 2, 4, 9, and 16 have appeared regularly in Mediterranean regions since 1998. When BTV-8 emerged unexpectedly in northwestern Europe in 2006, initially there were moderate morbidity and mortality rates in sheep and cattle, increasing in severity in the next 2 years, necessitating widespread vaccination programmes. Clinical signs in cattle with BTV-8 included fever, salivation, facial oedema, buccal and nasal mucosal lesions with ulceration, plus coronitis. BTV-8 also transmitted to the fetus via the placenta between 70 to 130 days of gestation at rates of 16% to 35%, resulting in hydranencephaly, occasionally accompanied by cerebellar dysplasia; porencephaly and hydrocephalus occurred. Surviving calves with hydranencephaly may show behavioural abnormalities, with dullness, inability to stand and suckle, disorientation, and impaired vision, similar to the 'dummy calves' observed in Australia due to AKAV infection. With increasing immunocompetence, fetal infection after 145 days of gestation results in viral clearance and a seropositive live calf. A recent review of the lesions in BTV-8 induced congenital malformations includes detailed description of the CNS lesions (Agerholm et al. 2015). Of note is that, as BTV-8 infections do not cause significant lesions to the brainstem and spinal cord, arthrogryposis and vertebral column malformations are not recorded. Australia currently has 10 serotypes of BTV (1, 2, 3, 7, 9, 15, 16, 20, 21, and 23) of low pathogenicity and occurring in cattle in tropical areas above the 'brevitarsis line' where sheep are rarely grazed. Mild clinical signs of BT in Australia have only been observed in sheep after experimental infections with some serotypes (e.g., 16 and 23) after movement of sheep to the northern endemic area.

Other Viruses

Rift Valley fever virus is an arbovirus in Africa that causes abortion in cattle, sheep, and goats after replication in the placentomes. A live attenuated vaccine used in endemic areas during outbreaks can cause congenital defects, including hydranencephaly, cerebellar hypoplasia, microcephaly, and arthrogryposis.

Wesselsbron virus, also in Africa, causes abortions and neonatal mortalities in lambs and kids with congenital malformations, including hydranencephaly and arthrogryposis.

Pigs

Japanese B encephalitis virus is a Flavivirus, and infection in midgestation of sows may cause abortion, mummification, stillbirths, and neonatal losses with CNS signs, although experimental infections have produced hydrocephalus, cerebellar hypoplasia, and hypomyelinogenesis.

Porcine herpesvirus 1 causes Aujesky's disease. When sows are infected in early gestation, embryonic resorption occurs, whereas in later gestation, abortion, stillbirth, mummification, and neonatal mortalities may occur.

Porcine parvovirus infection *in utero* produces different outcomes, depending on the stage of gestation. Embryonic mortality and resorption occurs from infection within the first month of gestation, whereas fetal death and mummification occurs from infections between days 30 to 70. Later infections may cause stillbirths.

Porcine respiratory and reproductive syndrome is caused by an Aterivirus that appears only able to cross the placenta in late gestation, causing abortions, stillbirths, mummification and neonatal losses.

Cats

Feline panleucopenia virus is a Parvovirus inducing fetal lesions dependent on the time of infection in gestation, ranging from embryonic death and resorption in early gestation, with cerebellar hypoplasia, retinal dysplasia, fetal death, and stillbirths from infections later in gestation.

Abnormalities Due to Uncertain Aetiologies, Including Fetal Monsters, Dropsies, Abortion, Maceration, and Mummification

Fetal Monsters

A fetal monster describes the abnormal presentation of a fetus in which the lesions displayed are considered sufficiently extreme for the fetus to be considered as 'monstrous'. The term is increasingly historical, having been considered in humans in biblical and medieval contexts, as resulting from 'unclean' practices. Interestingly, in animals a misshapen 'monk calf' born in Freiberg, Germany, in 1522 with limbs in extension and a fold of skin over the head (suggestive of arthrogryposis and a meningocoele) was compared with a monk and used to criticise Martin Luther, who responded that it represented the monstrosity of the Catholic church (Szabari 2006). However, with increasing scientific knowledge of the causes of congenital abnormalities and now elaboration of the pathological pathways that lead to the more extreme lesions occurring during development in humans and other animals, the term 'monster' is less commonly used as deformed animals are increasingly presented to veterinarians from owners seeking more rational explanations than the mystical or theological explanations of history. Unfortunately, many veterinarians have frequently dismissed congenital 'monsters' as idiopathic errors of development, contributing to the causes of dystocia or perinatal mortality, and rarely conducted the detailed investigations required to define the nature of the lesions presented.

However, as a number of these 'monster' entities have recently been found to be caused by inherited mutations, including several of the more common bovine fetal monsters recognised for over 100 years, there is emerging enthusiasm to better characterise them.

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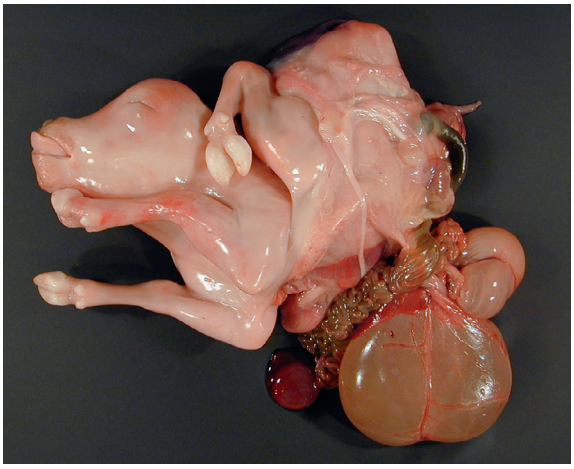
In recent years, the commonly recognised 'bulldog calves' affected with chondrodysplasia (OMIA 001271) and 'dropsy calves' affected with fetal hydrops or anasarca (OMIA 001562) have been characterised in Dexter and other breeds of cattle. 'Spinal deformity' calves characterised by shortening of the spine (see Fig. 9.16) and arthrogryposis (see Fig. 9.15) due to brachyspina (OMIA 000151) or congenital vertebral malformation (OMIA 001340) have been reported in Holstein–Friesian cattle. 'Curly calves' (see Fig. 9.8) with arthrogryposis multiplex (OMIA 001465), 'water head' calves with neuropathic hydrocephalus (OMIA 000487) causing a protruding encephalomeningocoele (see Fig. 9.9), and most recently, polymelia (see Fig. 9.7), or 'accessory limbs', with a variety of limb and other tissue developmental duplications (OMIA 002103) have been reported in Angus cattle. Similar descriptions of these disorders are increasingly recognised in other breeds and shown to be or suspected of being due to inherited disorders. Detailed description of these 'monsters' is provided in the inherited disorders section, and it is of great interest to investigators of congenital abnormalities, whether we will continue to see so-called 'idiopathic' fetal monsters in cattle and other species, ultimately determined to be mainly due to previously unrecognised inherited conditions. Already some forms of the spinal shortening deformity perosomus elumbis (OMIA 000789), the vertebral arch deformity spina bifida (OMIA 000933), and the spectacular deformity that almost invariably causes dystocia in cattle known as schistosomus reflexus (OMIA 000890) are considered more than likely to be caused by or associated with genetic factors, and thus provided with an OMIA number.

However, it is also plausible that other pathogenical pathways could also produce 'phenocopies' of these disorders should it be determined that they are inherited in certain breeds. Similar metabolic errors caused by different mechanisms of pathogenesis are well recognised in veterinary medicine. For example, alpha-mannosidosis in Angus cattle (OMIA 000625) and ingestion of the toxic plant *Swainsona glomerulifera* both inhibit cellular mannose metabolism, resulting in pathological accumulation of lysosomal storage material. Congenital deformities may arise from very different aetiological mechanisms, causing errors in the same or similar metabolic pathways, meaning both inherited and noninherited forms of these disorders may well be observed and need to be properly described. The following disorders are examples of deformities in cattle in which the evidence for their being transmitted by inheritance remains inconclusive.

Perosomus elumbis (PE; OMIA 000789) is a lethal congenital defect characterised by absence of the lumbar, sacral, and coccygeal spinal segments and spinal cord, associated with musculoskeletal malformations of the hindquarters. Bovine PE was first reported in the veterinary literature in 1832, and although there have been a number of recent case reports of the disorder in the Holstein–Friesian breed worldwide, investigations into a possible genetic aetiology of PE in cattle remain unreported (Agerholm et al. 2014). PE has also been reported in other animal species, including sheep, pigs, dogs, and horses, and although a recent publication suggested that fetal infection with bovine virus diarrhoea virus (BVDV) may have contributed to PE, plus an ovine PE case after feeding of pregnant sheep with *Veratrum californicum* on Days 16 and 17 of gestation. That teratogens should cause PE remains speculative and research into the aetiology of this disorder is required.

Spina bifida (SB; OMIA 000933) is a congenital defect characterised by defective closure of the two halves of the vertebral arch through which the spinal cord and meninges may or may not protrude. It is observed occasionally in cattle and not uncommonly in sheep, with inherited forms suspected in Poll Wiltshire and other breeds in Australia (Windsor et al. 2011a).



• **Fig. 9.22** Schistosomus reflexus of a fetus displaying spinal inversion, exposure of the abdominal viscera through a fissure of the ventral abdominal wall, and abnormal positioning of the limbs adjacent to the skull, with pulmonary and diaphragmatic hypoplasia.

Schistosomus reflexus (SR; OMIA 000890) is a fatal congenital disorder mostly observed in ruminants, characterised by spinal inversion, exposure of the abdominal viscera through a fissure of the ventral abdominal wall, limb ankylosis, abnormal positioning of the limbs adjacent to the skull, and pulmonary and diaphragmatic hypoplasia (Fig. 9.22). Variable components of SR include scoliosis, cleft sternum, exposure of thoracic viscera, and abnormalities of the digestive and urogenital systems. A 2005 report of a female Holstein SR calf with thoracoschisis, scoliosis, and anomalies of the appendicular skeleton also had abnormalities of the cardiovascular, respiratory, digestive, and urogenital systems. It was suggested that the SR occurred as early as the postgastrulation embryo and involved the intermediate mesoderm, with preliminary pedigree analysis indicating a possible genetic aetiology (Laughton et al. 2005).

Amorphous globosus, or acardiac monster, or fetal mole, is a spherical body attached to the fetal membranes of a normal fetus in the bovine (Fig. 9.23). They are formed from connective tissue surrounded by skin and may be of a different sex from that of the normal twin; although as there is not usually any gonadal development, they do not pose a threat of freemartinism (Long 2001).

Craniofacial deformities (CFDs) are relatively uncommon in animals, occurring sporadically, although with an interesting regularity in Australian sheep flocks. However, CFDs are widely prevalent in humans, accounting for approximately 75% of human birth defects, affecting 0.1% to 0.3% of all births and ranging from minor cosmetic irregularities to considerable structural malformations that can lead to problems in breathing and feeding, compromised social and mental development, and in extreme cases, death (Dwokin et al. 2016). As surgical reconstruction of CFDs is complex and often requires multiple procedures, understanding the mechanisms and aetiology of CFDs in animals is of potential importance in improving human CFD surgery. The most consistent denominator of this group is aplasia of the olfactory system and other cerebral hemispheric organisations (Szabo 1989). Lambs often present with agnathia (absence of the mandible) and misplaced ears (Fig. 9.24). Craniofacial defects include the following:

- ***Arhinencephaly*** characterised by marked reduction or complete absence of the olfactory organs, often involving ocular, nasal, and palatal defects. Where the cerebral hemispheres and lateral and

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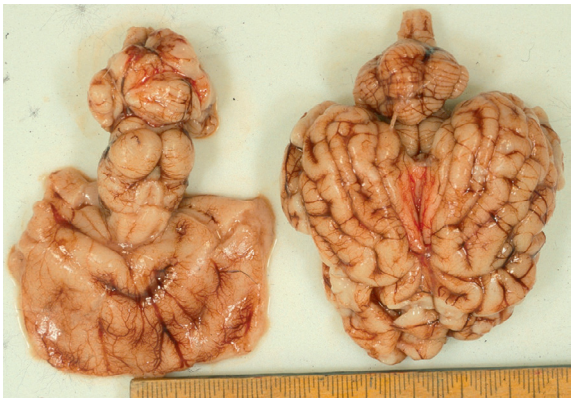
• **Fig. 9.23** Amorphous globosus or acardiac monster attached to the fetal membranes of a normal bovine, formed from connective tissue surrounded by skin.

• **Fig. 9.24** Craniofacial deformities with maxillary brachycephalus in an inherited form of congenital holoprosencephaly in a Border Leicester lamb from Australia.

third ventricles are reduced to a single structure with absence of the corpus callosum, the defect is termed *holoproencephaly.*

- ***Cebocephaly*** ('ape head') represents a transition between arhinencephaly and cyclopia, with two rudimentary eyes located in closely situated orbits, a flattened nose, frequently rudimentary and often with a single nostril, and brain malformations similar to cyclopia, sparing the cerebellum and brainstem.



• **Fig. 9.25** Congenital holoprosencephaly affecting the brain of a Border Leicester lamb with craniofacial deformities; note the single dysplastic cerebral hemisphere *(left)* compared with a normal brain *(right)*.

- ***Cyclopia*** is the most severe of these malformations, involving the orbits, brain, and nose, characterised by fusion of the eyes in a single orbital cavity with a proboscis above a rudimentary nose. The eyeballs may be normal in size, but anophthalmia and microphthalmia are common, and eyelids and lashes are usually absent. The anterior regions of the cerebrum, the septum pellucidum, the corpus callosum, and the cranial bones and nerves are either partially or completely absent. The cerebral hemispheres are completely fused and flattened. The thalamus and hypothalamus can be identified, but the third ventricle is usually absent, as are the olfactory bulbs, nerves, and the pituitary gland. Cyclopia is frequently accompanied by micrognathia or agnathia, cleft palate and harelip, spina bifida, polydactyly, sirenomelia, and defects of the heart and great vessels, kidneys, and adrenals.
- ***Otocephaly*** ('ear head') is related to cyclopia and arhinencephaly, occurring in varying grades of severity. In severe cases the forebrain and midbrain are usually affected and the third and lateral ventricles form a single cavity. The olfactory tracts are usually absent, the mouth and mandible are rudimentary, and the development of the tongue is incomplete. The eyes and ears are low set and fused or absent. In lower grades of otocephaly the brain may be normal but the eyes, ears, jaws, and nose are defective.

There is a particular interest in this group of congenital abnormalities in sheep, particularly because a necropsy study of 4408 newborn dead lambs in Australia from 79 predominantly Merino flocks found otocephaly in 25 lambs (Hughes et al. 1972), and a survey of congenital defects of sheep in Western Australia found that about 5% of the farmers had seen cyclopia in a large, predominantly Merino population of lambs (Dennis 1993). As mentioned, an inherited form of CFD has been described in Border Leicester sheep in Australia, with facial deformities (see Fig. 9.24) associated with holoprosencephaly (Fig. 9.25) of the cerebrum (OMIA 000478) and breeding studies suggesting autosomal recessive inheritance (Roth et al. 1987). Further, as previously discussed, in lambs born to ewes exposed to the teratogenic plant *Veratrum californicum,* CFDs and skeletal defects occurred with studies confirming embryonic vulnerability from Day 14. Findings confirmed that the severity of defects varied with level of toxicity and gestational stage, and identification of the toxins cyclopamine, cycloposine, and jervine enabled studies on CFDs in humans and other species with these alkaloids now known to interfere with Sonic hedgehog signalling.

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• **Fig. 9.26** Craniofacial deformities in a lamb from Australia characterised by otocephaly, with mandibular agnathia, ventral displacement of the ears, and an open trachea.

However, it would appear from the limited investigations possible thus far that neither inheritance nor exposure to known toxic plants or chemicals is able to explain the majority of sporadic yet persistent occurrence of CFDs in the Australian ovine population, usually characterised by otocephaly, with agnathia and ventral displacement of the ears commonly observed (Fig. 9.26). The possibility that at least some outbreaks of CFDs in Australian sheep may be due to arbovirus infections has been suggested (Ball 2011), although systematic studies are required to address a problem that could prove to be complicated by gene–environment interactions.

Numerous environmental factors are associated with the aetiology of CFDs in humans, including exposure to ionising radiation, vitamin deficiency, tobacco smoke, and maternal alcohol intake. However, it seems likely that human embryos exist with a genetic predisposition to CFDs due to sensitising mutations, such as the *Shh* genes that regulate craniofacial development, yet are influenced by environmental factors (Dwokin et al. 2016). Characterising these gene–environment interactions (GEI) could prove beneficial, particularly as identifying genetic mutations could provide prenatal diagnostic tools to limit the severity of human birth defects. With very few animal models of CFD susceptibility to environmental insult currently in existence, the identification of genes such as *Shh* in humans and other animals is important as it may influence future therapies of severe prenatal defects of human development and help ameliorate developmental defects in animal populations. That CFDs in Australian sheep frequently involve abnormalities of the jaw such as agnathia, and the *Shh* genes have an important role in craniofacial development, including the development of the jaws, suggests ovine CFDs offer a potentially important area for future study.

Dropsies

Dropsy is an old term for the swelling of soft tissues due to excessive accumulation of fluid, although similar to 'monster', the term is declining in use as the causes of these disorders are increasingly recognised. The three dropsical conditions of the conceptus commonly observed in veterinary obstetrics include oedema of the maternal placenta, the fetal placenta, and of the fetus. They may occur separately or in combination.

Oedema of the Fetal Membranes and Placenta

Placentitis caused by infections of the reproductive tissues often results in oedema of placenta, although this depends on the severity and distribution of the infection. The site of oedema may assist in characterising the nature of the infection. For example, in brucellosis in cattle caused by *Brucella abortus* infection, bacterial infection of the reproductive tissues may cause a severe necrotising placentitis and abortion, with oedema accompanying the necrosis causing partial destruction of the integrity of the caruncles, then spreading to the cotyledons and eventually to the intercotyledonary tissue of the fetal placenta (see Fig. 9.20), accompanying abortion. In contrast, in toxoplasmosis of sheep caused by the Apicomplexan protozoan *Toxoplasma gondii,* lesions are confined to the caruncles and cotyledons, with tissue hypertrophy (see Fig. 9.21) observed accompanying the proliferation of tachyzoites in endometrial cells and a higher chance of survival of the fetus to term.

Excessive quantities of fluid in the amnion or allantois, is referred to as hydramnios or hydrallantois, respectively. Hydrallantois appears more common than hydramnios, although fetal abnormalities are more likely to be associated with fluid accumulation in the amnion, as commonly occurs in chondrodysplasia (OMIA 001271) and 'dropsy calves' affected with fetal hydrops or anasarca associated with pulmonary hypoplasia (OMIA 001562) in Dexter calves. Although dropsy of the fetal sacs is more common in cattle and may be caused by a number of pathogenical pathways, including twinning (Harper et al. 1995, Windsor et al. 2006), it has also been observed in horses in pluriparous mares of 10 to 20 years of age between 7 to 9 months of gestation, in a dog litter, and on a number of occasions in sheep associated with twins or triplets, with up to 18.5 litres of fluid in the amniotic sac (Long 2001).

Apart from the hereditary cases of hydramnios that accompany the Dexter disorders and observed in other breeds from early to midgestation, hydrallantois of cattle is more likely to be seen in the last 3 months of gestation when there is a markedly accelerated production of allantoic fluid from 6 months of gestation. When placental dysfunction exists, as in abnormal placentation or placental circulation, the increase in fluid may become uncontrolled and lead to massive fluid accumulation. Hydrallantois has been associated with twinning in cattle, with a report of the birth of a single occurrence of hydrops fetalis that was accompanied by hydrops of the fetal sacs from a total of 514 twin calves born in the studies, suggesting a prevalence of 0.2% for bovine hydrops in experimental bovine twinning (Harper et al. 1995).

Hydrallantois cows present with progressive distension of the abdomen by the excessive fetal fluid. The volume of allantoic fluid may vary, with up to 273 litres imposing a serious strain on the cow, restricting respiration and impeding prehension and locomotion, with a gradual loss of condition leading to recumbency and death. Occasionally, affected animals may abort, with less severely affected cows achieving term in poor condition and presenting with dystocia from uterine inertia, often accompanied by incomplete dilation of the cervix. The diagnosis of bovine hydrallantois is based on the readily observed fluid distension of the abdomen in the last third of pregnancy, with confirmation obtained by the rectal palpation of the markedly swollen uterus and by the failure to palpate the fetus either per rectum or externally. The management of these cases depends on the viability of the cow, and moribund animals should be slaughtered. If the animal is viable and near term, Caesarean surgery is advised, performed with care to enable the fluid to escape slowly and prevent hypovolaemic shock. As hydrallantois is frequently seen in twin pregnancies in cows, it is important to search the grossly distended uterus for the

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second calf. Cases of hydrallantois that calve or are delivered by Caesarean surgery frequently retain the placenta and are at risk of metritis, with protracted convalescence and delayed conception. Use of a synthetic corticosteroid (20 mg dexamethasone) followed 4 to 5 days later by oxytocin administered by intravenous drip for 30 minutes is advised therapy (Long 2001). As the placenta is markedly oedematous, gross and microscopical examination of cotyledons and caruncles to identify if lesions of the endometrium are present is advised.

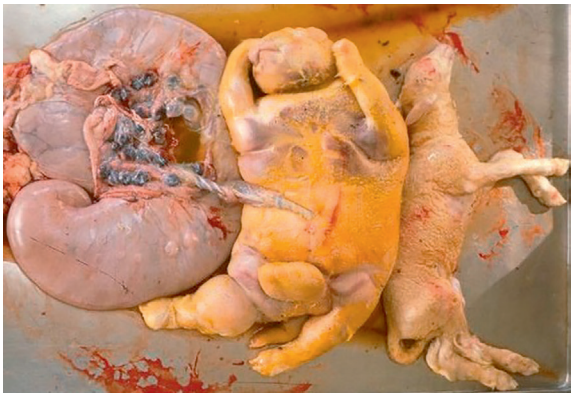
Dropsy of the Fetus

In humans massive oedema of the newborn infant has been recognised for at least three centuries, and a causal relationship with maternal–fetal blood group incompatibilities was recognised soon after red cell antigens were identified. Fetal hydrops was, historically, mostly attributed to severe maternal isoimmunisation to fetal blood group antigens foreign to the mother. However, in the majority of current human cases, the diagnosis of hydrops fetalis is now made prenatally, with few due to immune mechanisms and many causes for nonimmune hydrops now recognised, including: haematological disorders; infections; chromosomal abnormalities; tumours; and congenital anomalies, including pulmonary hypoplasia. The pathophysiological events in fetal hydrops were poorly understood until recent studies in sheep models of fetal hydrops induced by occlusion of lymphatic drainage, obstruction of cardiac venous return, and induced fetal anaemia or tachycardia (Andres & Brace 1990). However, of the numerous physiological disturbances noted in human fetal hydrops, a three- to fivefold increase in atrial natriuretic peptide (ANP) has been noted to accompany both human fetal hydrops with cardiac anomalies or isoimmunisation, as well as ovine hydrops induced by obstruction of venous return, sustained tachycardia, or induced anaemia. As vascular permeation of albumin is enhanced and cardiovascular and renal homeostatic adaptations are influenced by ANP, a key role for ANP in fetal hydrops has been suggested, particularly as ANP levels return to normal with resolution of the hydrops (Shinbane et al. 1997).

In animals, there are also several types of fetal dropsy of obstetrical importance, including fluid accumulation in the fetal brain (hydrocephalus and hydranencephaly that may require intervention, as previously described), in the abdomen (ascites), and in body cavities and subcutis (anasarca). The form of the fetus and the degree of obstetrical hazard are determined by the location and amount of the excess of fluid, with dystocia risk due to the increased diameter of the fetus.

Fetal ascites involves excessive fluid in the peritoneal cavity and frequently accompanies fetuses that have died preterm as a result of decomposition and may cause dystocia, infectious disease of the fetus, and some developmental defects where there is compression of the peritoneal cavity. For example, fetal ascites and hydramnion commonly occur in chondrodysplasia in the Dexter and other breeds of bulldog calf, when there is insufficient space to accommodate the viscera, and the calf presents with a large ventral hernia (see Fig. 9.13). Fetal ascites as a cause of dystocia is usually assisted by incising the fetal abdomen with a fetotomy knife.

Fetal anasarca is a common event when the fetus is carried to term but there is lack of progress in second stage parturition, leading to an increase in fetal volume from excessive fluid in the subcutaneous tissues, particularly of the head and hindlimbs. Tissue swelling can be extreme, and there is frequently excessive fluid in the peritoneal and pleural cavities with dilatation of the umbilical and inguinal rings as well as hydrocele. Hydramnios can also be extreme, and there may also be some hydrallantois present.



• **Fig. 9.27** Hydrops fetalis in a lamb displaying severe oedema of subcutaneous tissues, fluid accumulation in the serous cavities, and oedema of the placenta, accompanying a normal twin lamb *(on right)*; hepatic extramedullary haemopoiesis suggested fetal anaemia.

Animals with fetal anasarca should be necropsied as the anasarca may be attributed to developmental lesions. Fetal anasarca associated with pulmonary dysplasia resulting in severe *cor pulmonale* is now well recognised in Dexter calves (OMIA 001562) and some other breeds at term (Windsor et al. 2006). The fetal hydrops in the Dexter calves with pulmonary hypoplasia appears to have been a progressive development *in utero* in late gestation, leading to fetal dystocia and associated with development of hydramnion, resulting in death of the calves at the onset of parturition, unlike in Dexter 'bulldog' fetuses that are often spontaneously aborted in midgestation. Further, an outbreak of fetal anasarca in Poll Dorset and Dorset sheep from ewes with hydramnion was reported in association with suspected fetal anaemia (Plant et al. 1987). Ewes showed marked abdominal distension, and most died at parturition and were unable to deliver the large affected fetuses weighing up to 18 kg and displaying severe oedema of subcutaneous tissues (Fig. 9.27), fluid accumulation in the serous cavities, and oedema of the placenta (OMIA 000493). As hepatic tissue from affected lambs displayed generalised extramedullary haemopoiesis and oedema on microscopical examination, the findings were hypothesised to resemble those of the homozygous a-thalassaemia in infants associated with haemoglobin Barts. Ovine fetal hydrops has also been reported in Awassi and Beulah Speckle Face and Cheviot sheep, with five Cheviot and Cheviot–Texel crossbred lambs born with pulmonary hypoplasia (Alleaume et al. 2012).

Abortion, Maceration, and Mummification

Fetal death may be followed by abortion, maceration, or mummification, with abortion and maceration occurring when endocrine support of pregnancy is lost. Abortions may be recognised by the presence of the fetus(es) or/and an abnormal vaginal discharge from the dam. Maceration occurs when the fetus fails to abort, and decomposition *in utero* occurs, resulting in retention of the fetal skeleton in the reproductive tract compromising future breeding. Palpation and/or ultrasound will usually result in a positive diagnosis.

In fetal mummification, the fetal death is rarely apparent, and there is no discharge or signs of return to oestrus, with owners recognising that the animal is failing to develop the expected external signs of late pregnancy. Fetal mummification occurs occasionally in most domestic species, although it is considered more common in multiparous and polytocous species such as pigs. It is likely that many different pathogenical mechanisms are responsible for early

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fetal mortality as this outcome is unpredictable and influenced by several factors, including: the cause of fetal mortality; differences in pregnancy between species; stage of gestation at fetal death; and the number of fetuses, as reviewed recently (Lefebvre 2015). In cattle fetal mummification has an incidence of less than 2%, with breed and previous occurrence as risk factors; there is a higher incidence of fetal mummification in Guernsey and Jersey cattle and a higher risk of recurrence (30%) in cows that have experienced a similar event in a previous gestation. Mummification occurs most often in cattle between the third and eighth month of gestation, without any concomitant luteolysis of the corpus luteum (CL) or opening of the cervix. Fetal mummification associated with a persistent CL is observed mainly in cattle and goats, as both species are dependent on progesterone (P4) produced by the CL for the maintenance of pregnancy. However, in cattle the placenta can produce sufficient P4 to maintain pregnancy between Days 150 to 200 of gestation. After fetal death, the amniotic and allantoic fluids are resorbed, fetal tissues and annex membranes dehydrate, and, eventually, the caruncles disappear. The longer the mummified fetus is retained, the dryer, firmer, and more leathery the tissues of the fetus become.

Potential causes for bovine fetal mummification include: infections (BVDV, leptospirosis, *Neospora caninum,* fungal infections); mechanical factors (compression and/or torsion of the umbilical cord); uterine torsion; defective placentation; some genetic abnormalities causing fetal loss; abnormal hormonal profiles; and chromosomal abnormalities. In practice, a definitive aetiology is rarely established with tissue decomposition and autolytic changes compromising histological, microbiological, and chromosomal analyses, although development of molecular tests is increasing the opportunity for some diagnoses to be established if DNA extraction occurs.

The majority of diagnoses of suspected fetal mummification (63%) are established by veterinarians after the owner notices that the cow's abdomen is unusually small for the given stage of pregnancy and that no significant systemic or other illness is observed in cows except for spontaneous abortion, which may or may not occur. The diagnosis of fetal mummification is generally uncomplicated, by transrectal palpation and ultrasonographic examination revealing that the mummified fetus is a compact, firm, and immobile mass without placental fluid or placentomes. Ultrasound reveals the absence of a heartbeat, and the general physical examination of the dam usually indicates other systems appear normal. A systematic approach to treating pregnant cows diagnosed with fetal mummification is suggested (Lefebvre 2015), with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) considered most effective in expelling the fetus. After expulsion of the fetus, a uterine lavage may be performed to remove fetal and placental debris, and the cow can be rapidly induced into oestrus and inseminated. In the absence of a treatment response, the most cost effective option is then to proceed with a second injection of $PGF_{2\alpha}$.

Further information on fetal mummification in the major domestic species, including common aetiologies, and treatment options for restoring future pregnancy and herd fertility can be found in the recent review (Lefebvre 2015).

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10

Prolapse of the Cervix and Vagina

DAVID E. NOAKES

Typically, prolapse of the cervix and vagina (CVP) is a disorder of ruminants, normally in late gestation. Occasionally, it is seen after parturition and rarely occurs unconnected with pregnancy or parturition. It less frequently occurs in pigs. It can be recognised by the protrusion of varying parts of the vaginal wall and sometimes the cervix through the vulva, so that the vaginal mucosa is exposed. In some bitches, hyperplasia of the vaginal mucosa occurs at prooestrus/oestrus, which may protrude through the vulva. This is sometimes referred to as vaginal prolapse, although such a description is incorrect and is not comparable with the condition in other species (see Chapter 33).

Sheep

Incidence and Cost

The disorder is much more common in sheep than in other species and is thus of real economic importance. Average incidences are 0.53% (Edgar 1952), 0.46% (Bossé et al. 1989), 0.98% (Low & Sutherland 1987), and 1.05% over 2 years (Jackson et al. 2014), which may rise to 20% and 46% in some flocks. In any 1 year there can be considerable between-farm variations; studies involving 113 and 88 farms in the years 2000 and 2001 in the Southland (S) and Hawkes Bay (HB) regions of New Zealand showed the annual incidence for both years was 0% to 3.9% for the HB farms and was 0% to 5.9% for the S farms. The financial cost of CVP is substantial. For example, Hosie (1989) calculated that in the United Kingdom each case cost £41, based on the average cost of replacing a breeding ewe minus the value of a culled affected ewe, lamb mortality, and the cost of treatment. In addition, there are the unquantifiable welfare implications. The financial costs of the disorder are based on these assumptions:

- Some affected ewes will die.
- If not treated, some will abort.
- Some will have reduced fertility.
- Ewes may be culled because of the possibility that prolapse may recur.
- The stillbirth rate and neonatal mortality are increased.
- There is a greater chance of dystocia in affected ewes.

Classification of Severity

The disorder is normally easily recognised, although sometimes the prolapsed organ can be confused with the allantochorion as it protrudes from the vulva before it ruptures. The severity of the disorder varies, and a number of different classifications have been used. Probably the simplest is that used by Bosse et al. (1989), as illustrated in Figs 10.1 and 10.2 which is:

- Stage 1, in which the vaginal mucosa protrudes from the vulva when the ewe is recumbent but disappears when she stands;
- Stage 2, in which the protruding vaginal mucosa remains visible even when the ewe stands; the cervix is not visible; and
- Stage 3, in which the vagina protrudes and the cervix is visible.

Other systems of classification have been used that also take into account the duration of the prolapse, its size, and the other organs contained within the prolapsed organ. Cox (1987) uses the terms mild, moderate, and severe. The bladder is most frequently involved, as it becomes reflected to occupy the vesicogenital peritoneal pouch. This can be followed by complete or partial constriction of the urethra, causing urinary retention; the uterine horns and intestines can also be involved. Real-time ultrasonography can be used to diagnose the contents of the prolapse (Scott & Gessert 1998).

Causes and Pathogenesis

Much of the evidence relating to the cause of the disorder is anecdotal and often contradictory. In the New Zealand study by Jackson et al. (2014), the crude relative risk of a prolapse occurring was 5.3 times higher in ewes carrying twins and 11.3 times higher in those carrying triplets than with singletons. It was less common in pure or crossbred Perendale ewes, which is evidence of a breed predisposition, whereas it was more prevalent in farms with a moderate to steep terrain compared with a flat terrain. There are probably a number of predisposing factors, some of which may interact, although for the prolapse to occur, there must be some inherent defect in the structure of the vaginal wall. Predisposing factors include:

- Hormonal excesses and imbalances,
- Hypocalcaemia,
- Large fetal load (twins or triplets),
- Fat condition,
- Thin condition,
- Inadequate exercise,
- Short tail docking,
- Bulky food (root crops),
- Excess dietary fibre,
- Dietary oestrogens and their precursors,
- Sloping terrain,
- Vaginal irritation,

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• **Fig. 10.1** Moderate, early prolapse (stage 2) of the vagina in a ewe.

• **Fig. 10.2** Severe prolapse (stage 3) of the vagina in a ewe.

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- Previous dystocia,
- Inherited (breed) predisposition.

Irrespective of the alleged predisposing factors, there are three requirements for CVP to occur (McLean 1956). They are the following:

- The vaginal wall must be in a state in which it can be readily everted, and the vaginal lumen must be large.
- The vulva and vestibular wall must be relaxed.
- There must be a force that can displace the vaginal wall, causing it to become everted.

Studies by Ayen et al. (1998) have attempted to measure the changes in the compliance of the vaginal wall (which will be related to the ease with which the wall can be everted) and the size of the vaginal lumen, the capacity of the vagina (which will assess the ease with which the everted vagina can be expelled to the exterior). There are considerable between-animal variations in these parameters, which explain why CVP occurs in some ewes but not others. The compliance of the vaginal wall was greater during the second than the first pregnancy, and this supports the observation that CVP is less frequently identified in primigravid than in plurigravid ewes. Both endogenous and exogenous estradiol and progesterone can influence the compliance of the vaginal wall and the capacity of the vagina. Ennen et al. (2011) found that peripheral plasma progesterone concentrations of ewes with vaginal prolapse were higher than in unaffected controls (19.35 vs 10.44 ng/mL), whereas oestradiol values were not statically different. The vaginal wall is comprised of collagen and smooth muscle, so both parameters will be influenced by either the amount, the distribution, or the type of collagen present (Ayen & Noakes 1998). There is evidence that there are changes in the tissue composition of the vaginal wall that influence its function, as assessed by in vitro tensiometry (Ulrich et al. 2014). The vaginal wall of pregnant ewes was the most extensible but the weakest, whereas tissues from both nulliparous and parous nonpregnant ewes were strong and elastic. Extrapolation from work relating to genitourinary prolapse in humans suggests that it may well be a change in the collagen type that reduces the mechanical strength of the vaginal wall (Jackson et al. 1996). Studies of human patients with prolapse showed that the vaginal wall had more total collagen, particularly subtype III, as well as greater active expression of the matrix metalloproteinases 9, compared with normal controls (Moalli et al. 2005). This, the authors considered, was an indication of active tissue remodelling occurring. Furthermore, the influence of oestrogens on vaginal collagen metabolism was shown in a human study in which estradiol valerate therapy in menopausal women caused a significant reduction in total aged collagen but an increase in new collagen (Jackson et al. 2002). In prolapsed tissue, the alpha chain of collagen 1 showed a decrease expression compared with unaffected ewes in late pregnancy. Biomolecular analysis shows that ewes suffering from vaginal prolapse have periparturient changes in metabolism of vaginal connective tissue, for example, collagen (Ennen et al. 2011).

Clinical Signs and Progression of the Disease

The clinical signs are obvious that the only possible confusion might be the protruding allantochorion. Prolapse occurs most frequently in the last 2 to 3 weeks of gestation. The only distinction that needs to be made is the severity. In sheep a severe prolapse with heavy straining is not well tolerated, and death from shock, exhaustion, and anaerobic infection is common. Abortion or premature delivery, often of a dead fetus, may be followed by rapid maternal recovery. In a study involving 129 ewes with CVP, spontaneous parturition occurred in 26%, with dystocia in 58%, of which 70% suffered from incomplete cervical dilatation ('ring womb') (Kloss et al. 2002).

White (1961) first described a fatal condition of heavily pregnant ewes in which the intestines become prolapsed through a spontaneous rupture of the dorsal or lateral wall of the vagina. This has been shown to be associated with vaginal prolapse, although why it should occur is not entirely clear (Knottenbelt 1988). A study involving the Norwegian Dala breed has questioned this theory and has presented information suggesting that it might be due to a number of reasons, including uterine torsion (Mosdol 1999).

Treatment

The early replacement and retention of the prolapse is very important to prevent trauma and to ensure that the ewe maintains pregnancy to term. Hosie (1989) reported on the consequences of such a prolapse on the health and viability of both ewes and lambs. Of the 183 ewes, 9.2% suffered from vaginal rupture, and a similar number had other unspecified complications, whereas 14.6% of the 253 lambs were either stillborn or died soon after birth.

Treatment of ewes with CVP is often carried out without any consideration for the welfare of the animal. It is relatively easy for veterinarians to reduce pain and discomfort during replacement by using caudal epidural anaesthesia. The technique, which is described in Chapter 13, should be used for all but the mildest prolapse; it also makes the replacement much easier to perform.

In sheep the perineal wool or string fastened to it may be tied across the vulva; large safety pins have sometimes been used. For retaining the prolapsed vagina of ewes, Fowler and Evans (1957) and Jones (1958) first described the use of a stainless steel stay in the form of the letter U, which is placed in the vagina. The emerging ends are bent at right angles and fitted with eyes that are securely fastened with string on either side to the wool of the gluteal region. This type of prolapse retainer has been improved by the development of a plastic spoon, which is fastened in the same way as the U-shaped device, or by combining it with a harness made from baling twine or nylon strapping (Fig. 10.3). Buhner's method, which is described in detail in the section on cattle later in this chapter, is equally applicable to ewes with a prolapsed vagina; it involves the laying of a suture of tape subcutaneously, using a large half-curved cutting suture needle; the smaller (15 cm long Buhner's needle) which is a smaller version of the one used in cows is preferable (see Fig. 10.6).

Insofar as it is possible that there may be a genetic predisposition to the disorder, it would seem unwise to breed from animals that have previously suffered from vaginal prolapse. There is little doubt that by adopting this culling policy over the years, stock owners have exerted a large measure of control over the condition. Although the recurrence rate is variable, Piorowicz (1987) and Hosie (1989) quoted recurrence rates of 72% and 40% respectively; an 18% rate in a small study involving 11 ewes was reported by Ayen and Noakes (1997).

Cattle

Causes and Pathogenesis

The exact cause of the disorder has not been ascertained but several factors are generally believed to play a part. Cattle of the beef breeds, particularly Herefords, Simmentals, and Charolais, are

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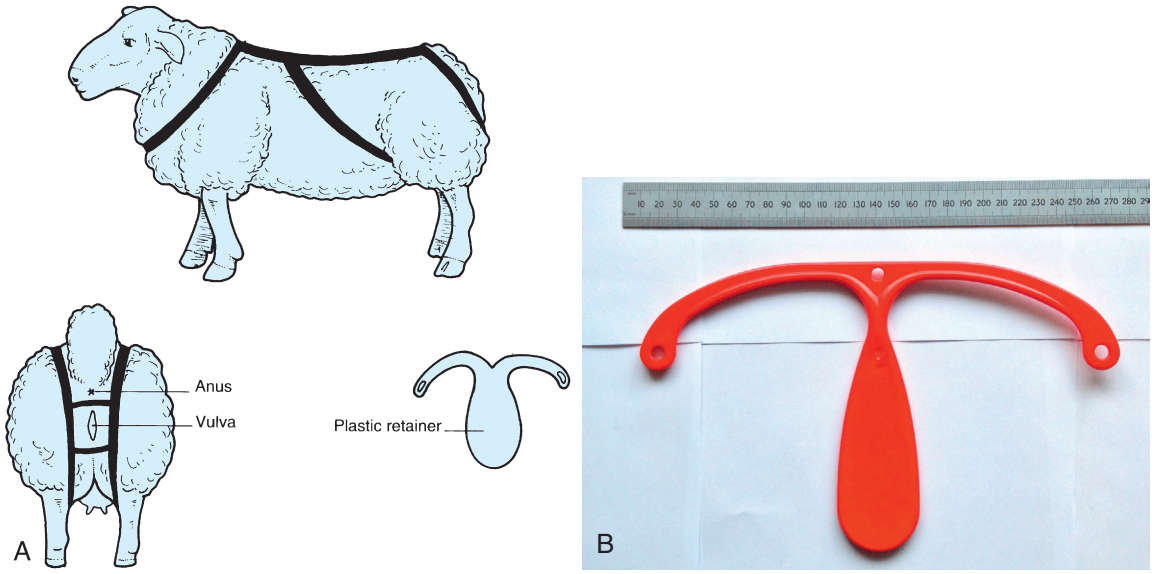

• **Fig. 10.3** (A) The positioning of a harness (constructed of baling twine or nylon strapping) in a ewe together with a plastic retainer. The latter is not always used if pressure from the harness on the perineum is sufficient to retain the prolapse. (B) The plastic retainer. Plastic prolapsed retainer normally used with harness but can be used with cotton tape tied to wool. (Courtesy Will Douglas, 'Nettex'.)

most commonly affected. Woodward and Queensberry (1956) recorded 1.1% of vaginal prolapses in 7859 pregnancies in Hereford cattle in the United States, and it has been suggested that in this breed the anatomical anchorage of the genital tract is less efficient than in other animals. An excessive deposition of fat in the perivaginal connective tissue and ligamentous relaxation may increase the mobility of the vagina. Both these effects might be due to a state of endocrine imbalance, in which oestrogenic hormones predominate; the administration of diethylstilbestrol has been shown to soften the suspensory ligaments of the genital tract. When oestrogenic substances are present in inordinate amounts in the diet, as in subterranean clover pastures of Western Australia (Bennetts 1944) or in mouldy maize and barley, which are considered to have a high oestrogen content, this can result in a high incidence of prolapse. When heifers are fed these in their diet, they may show vulvovaginitis, with oedema of the vulva, relaxation of the pelvic ligaments, tenesmus, and vaginal prolapse (Koen & Smith 1945, McErlean 1952). Predisposition to CVP is inherited, as shown by its greater frequency of occurrence in beef cattle. Mechanical factors, such as the increasing intraabdominal pressure of late pregnancy and gravity acting through the medium of a sloping byre floor when cattle are tethered, are considered to be significant (McLean & Claxton 1960).

Postparturient prolapse of the vagina of cattle is usually due to severe straining in response to vaginal trauma or infection after a serious dystocia. Vaginal contusion at parturition, followed by *Fusobacterium necrophorum* infection, exerts a high degree of irritation with frequent exhausting expulsive efforts.

Clinical Signs and Progression of the Disease

Initially, the lesion involves a protrusion of the mucous membrane, more frequently the floor of that part of the vagina that lies just cranial to the urethral opening. In severe cases the whole of the anterior vagina and cervix may protrude. The earlier in pregnancy the disease develops, the more severe it is likely to become because advancing pregnancy tends to accentuate the condition. Most cases occur in the last 2 months of gestation. In the mildest form the prolapse appears only when the cow is recumbent, and when the animal rises, it recedes. However, the tendency is for the prolapse to become more severe so that, in time, a large tissue mass protrudes and does not disappear in the standing position. The protruding tissues, with their circulation impaired, are prone to injury and infection. The resultant irritation causes expulsive straining efforts. This increases the severity of the prolapse, and a vicious circle is established. Eventually, the whole of the vagina, cervix, and even the rectum may become everted (Figs 10.4 and 10.5). Thrombosis, ulceration, and necrosis of the prolapsed organ, accompanied by toxaemia and severe straining, lead to anorexia, rapid deterioration in bodily condition, and occasionally death.

Treatment

If prompt attention is given, simple measures often succeed. The aim is to arrest the process by early replacement and retention of the prolapsed portion. Caudal epidural anaesthesia (see Chapter 13) is indicated both to obviate straining and to desensitise the perineum for suturing. The everted mass is cleaned, using plain water or a mild nonirritant antiseptic, and replaced gently with the palm of the hand, being careful not to cause trauma to the inflamed and sometimes fragile tissue. It is retained by tape or stout nylon sutures that cross the vulva and are inserted in the perineal skin. Quill sutures tied over rubber tubing are best. In cases in which the vagina has suffered little damage and especially in which parturition is imminent, such measures are usually sufficient. However, when irritation from trauma and infection causes vigorous straining, or even the trauma caused by the insertion of the sutures themselves, the retaining sutures may tear through the tissue, become displaced, and prolapse recurs. Although straining may be controlled by caudal epidural anaesthesia in the short term and although xylazine will prolong its effect (see Chapter 13), it is not practical to provide continuous anaesthesia by this means. Perineural injection of the pudic nerves has the same effect and the same disadvantage. Tenesmus has been prevented for several

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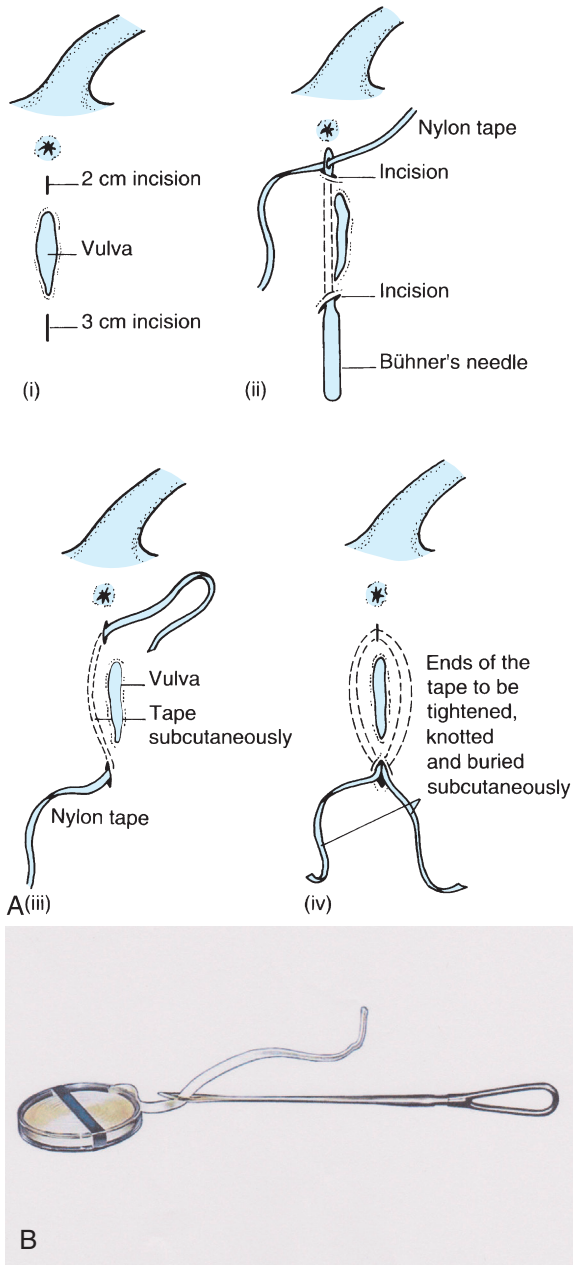
• **Fig. 10.4** Prolapse of vagina in a Charolais cross cow at 7 months of gestation. (Courtesy the late Professor Barrie Edwards.)

• **Fig. 10.5** Prolapse of vagina, cervix, and, because of persistent straining, the rectum.



• **Fig. 10.6** (A) Buhner's method for the retention of vaginal prolapse. (B) A 30 cm Buhner's needle suitable for use in cow with reel of nylon tape. (Courtesy R. J. Mathews, Massillon, Ohio.)

days and for up to a week or more by the induction of an artificial pneumoperitoneum.

Because of the deficiencies of the simple suture methods of retention, in the author's experience the best means of retaining the replaced vagina is the technique described by Buhner (1958) (Fig. 10.6). It entails the placing, by use of a special needle, of a subcutaneous suture of nylon tape around the vulva. To facilitate introduction of the large needle, two stab incisions are made (under epidural anaesthesia previously induced to aid replacement of the vagina) in the midline; the upper one is midway between the dorsal commissure of the vulva and the anus, and the lower one is immediately beneath the ventral vulvar commissure. The needle is inserted into the lower incision and gradually passed subcutaneously up the right side of the vulva until its point emerges through the

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• **Fig. 10.7** Chronic prolapse of the cervix, treated by using Robert's modification of Caslick's operation.

upper incision, whereupon the needle is threaded with a double length of nylon tape. While one end of the tape is firmly held, the loaded needle is pulled downwards until free of the lower incision, when it is unthreaded, thus leaving a length of tape protruding from each incision. The needle is now inserted again into the lower incision and passed subcutaneously up the left vulvar labium. When its point emerges the needle is threaded, then pulled backwards and outwards from the lower aperture and unthreaded. The tape now encircles the vulva subcutaneously, and its two ends hang from the lower incision. These ends are tied with a simple knot with such a degree of tightness that four fingers can be inserted flatwise up to their second joints into the vulva. The upper incision is closed with a couple of sutures of fine monofilament nylon, and the lower incision can be either left open or sutured according to the cow's proximity to parturition. The suture causes practically no tissue reaction, the vulvar labia are not damaged by it, and it can remain in situ for months until the cow is on the point of calving, when the knot should be cut so as to release the thread and allow the vulva to dilate for the birth of the calf.

For cows showing recurrent prolapse that are remote from parturition and also for postpartum cases, Roberts (1949) suggested a method of almost complete surgical occlusion of the vulva by a technique that is really an extension of Caslick's vulvoplasty operation for preventing vaginal aspiration in mares (Fig. 10.7). Under caudal epidural or local infiltration anaesthesia, strips of mucous membrane, 1.2 cm wide, are dissected from the upper three-fourths of each vulvar lip. The denuded areas are then approximated by means of fine, nonabsorbable sutures, and a few mattress sutures of tape or stout nylon are deeply placed across the vulva to protect the coapted lips from the effects of straining. First intention healing should occur, and the suture line must be incised when parturition is imminent.

Farquharson (1949), who saw hundreds of cases of vaginal prolapse in Hereford range cattle in Colorado, successfully applied a technique of submucous resection, or 'reefing' operation, on the prolapsed organ. The object of the operation, which should not be performed later than 3 to 4 weeks from term, is to excise the protruding mucosa, which forms the bulk of the everted mass, and then approximate the cut edges. The operation is performed under caudal epidural anaesthesia. Subsequent parturition and conception are not affected, and the cure is permanent. An alternative approach is to prevent displacement of the cervix caudally by anchoring it, using nonabsorbable sutures or nylon tape, to the prepubic tendon or the sacrosciatic ligaments (Winkler 1966). Although Winkler describes the technique as being done under caudal epidural anaesthesia, in the author's experience, although the approach to the sacrosciatic ligament is possible using this approach, attachment to the prepubic tendon requires general anaesthesia, with the cow in dorsal recumbency, and a caudal midline laparotomy between the two udder halves.

Pigs

Vaginal prolapse (Fig. 10.8) occurs in gilts at oestrus, but more frequently in housed pregnant older sows (> 5 pregnancies) that are in fat bodily condition, carrying large litters and with high levels of

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• **Fig. 10.8** Vaginal prolapse in a sow. (Courtesy of Professor Olli Peltoniemi.)

feed intake; anecdotally, the feeding of mouldy cereals, supposedly because of their high oestrogen content, has been implicated. An incidence of 0.5% has been quoted. In mild cases the prolapse is often intermittent, with it protruding only when the sow is recumbent and disappearing from view when she stands. However, if it continues to protrude, a simple tape or Buhner's suture (as described in Fig. 10.6) can be used but obviously must be cut as farrowing approaches. Affected sows/gilts should be culled. Vaginal prolapse in pigs is discussed in greater detail in Chapter 17.

Dogs

Vaginal hyperplasia occurs in bitches around the time of oestrus, and when there is excessive oedema of the vaginal mucosa cranial to the urethral orifice, it can protrude from the vulva appearing as if the vaginal wall has prolapsed (Johnston et al. 2001); thus this is sometimes incorrectly referred to as vaginal prolapse (see Chapter 34). A few cases of true vaginal prolapse have been reported, both single case reports (Memon et al. 1993, Alan et al. 2007). One case was first seen around the time of oestrus and recurred during pregnancy, whereas the other first appeared in late gestation and resulted in dystocia. Chronic prolapse during pregnancy in the bitch has been reported (Memon et al. 1993, Alan et al. 2007) that was treated by hysteropexy, and subsequent caesarean followed by ovariohysterectomy, respectively. Long-term, chronic vaginal prolapse in the absence of pregnancy involving herniation of abdominal viscera has also been described (McNamara et al. 1997).

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PART 3

Obstetrics and Surgery of the Reproductive System

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11

Approach to an Obstetrical Case

TIMOTHY J. PARKINSON, JOS J. VERMUNT AND DAVID E. NOAKES

Each case of dystocia is a clinical problem that may be solved if a correct procedure is followed. The successful management of the case requires obtaining a history from the owner or attendant, a focused clinical examination, and specific examination of the reproductive tract. There should be careful differentiation between a labour that is not fully established from one that has become interrupted, and a careful diagnosis should be made of any faulty fetal disposition before attempting any correction. There should also be a differentiation between whether the labour has been interrupted due to a problem with the birth process per se, and whether other disease processes are interfering with it.

Causes and Classification of Dystocia

A normal birth occurs when the **expulsive forces** are sufficient to propel a **fetus of normal size and disposition** through a **birth canal of adequate size**. Dystocia occurs when any of these are abnormal or insufficient. Thus the causes of dystocia can be considered in terms of the:

- Expulsive forces
- Adequacy of the birth canal
- Size and disposition of the fetus

Difficult birth will occur when the expulsive forces are insufficient, when the birth canal is of inadequate size and shape, or when the presenting diameter of the fetus is unable to pass through the normal birth canal because it is too large or its disposition prevents it from doing so. An overview of the types and causes of dystocia are given in Fig. 11.1.

Defects of the expulsive forces and of the adequacy of the birth canal are commonly referred to as **maternal dystocia**, whereas those due to defects of the presentation of the fetus or to its disproportion (i.e., with respect to the size of its dam) are known as **fetal dystocia**. However, there will be times when it can be difficult to identify the primary cause and others when there will be a change in the dominant cause during the course of the dystocia.

Defects of Expulsive Forces

Defects of expulsive forces generally refer to intrinsic defects of uterine contractility, nervous voluntary inhibition of labour, or failure of contraction due to mineral/hormonal imbalances (**primary inertia**), or to exhaustion of either the uterine muscle itself or depletion of pituitary oxytocin stores (**secondary inertia**). Failure of contraction of abdominal muscles during second stage labour can also occur.

Defects of the Adequacy of the Birth Canal

The birth canal consists of the internal tubular organs of the reproductive tract from the uterine horns and body to the vulva. Abnormalities of any part of these structures can lead to dystocia.

Such abnormalities include function disturbances of the genitalia (e.g., incomplete cervical dilation; torsion of the uterus), obstructions (e.g., neoplasia), or mechanical abnormalities (e.g., double cervix; remnants of the paramesonephric ducts). Rarely, the uterus can be displaced into a subpubic position or through a hernia in the abdominal wall.

Abnormalities of the surrounding tissues can, likewise, lead to dystocia. Such tissues include the pelvis (e.g., heritable pelvic malconformation such as occurs in Belgian Blue cattle and brachycephalic dog breeds; fractures of the pelvic bones; sacroiliac dislocation), the bladder (e.g., prolapse into the vagina), or excess intrapelvic fat,

Faulty Fetal Disposition

During pregnancy, particularly in monotocous species such as cattle, horses, sheep, and goats, the fetus assumes a disposition within the uterus which is such that the space occupied is as little as possible: for example, the vertebral column and limbs will be flexed to varying degrees. In order to negotiate the birth canal unaided, these joints need to be extended. The term 'faulty' or 'abnormal fetal disposition' is used to describe the situation in which the fetus has failed to assume the disposition that enables it to be expelled unaided *per vaginam.* The universally accepted terminology to describe these abnormalities is that first defined by Benesch. This involves the use of the terms presentation, position, and posture, each of which has a specific meaning in relation to veterinary obstetrics (Fig. 11.2).

Presentation signifies the relationship between the longitudinal axis of the fetus and the maternal birth canal. These may be:

- **Longitudina**l presentation, which can be **anterior** or **posterior**, depending on which fetal extremity is entering the pelvis;
- **Transverse** presentation; **ventra**l or **dorsal**, according to whether the dorsal or ventral aspect of the trunk is presented; and
- **Vertical** presentation; **ventral** or **dorsal**. Vertical presentations are very rare, and only the obliquely vertical 'dog-sitting' presentation in the horse needs to be considered in detail.

Position indicates the surface of the maternal birth canal to which the fetal vertebral column is applied. It can be **dorsal**, **ventral,** and **left or right lateral**.

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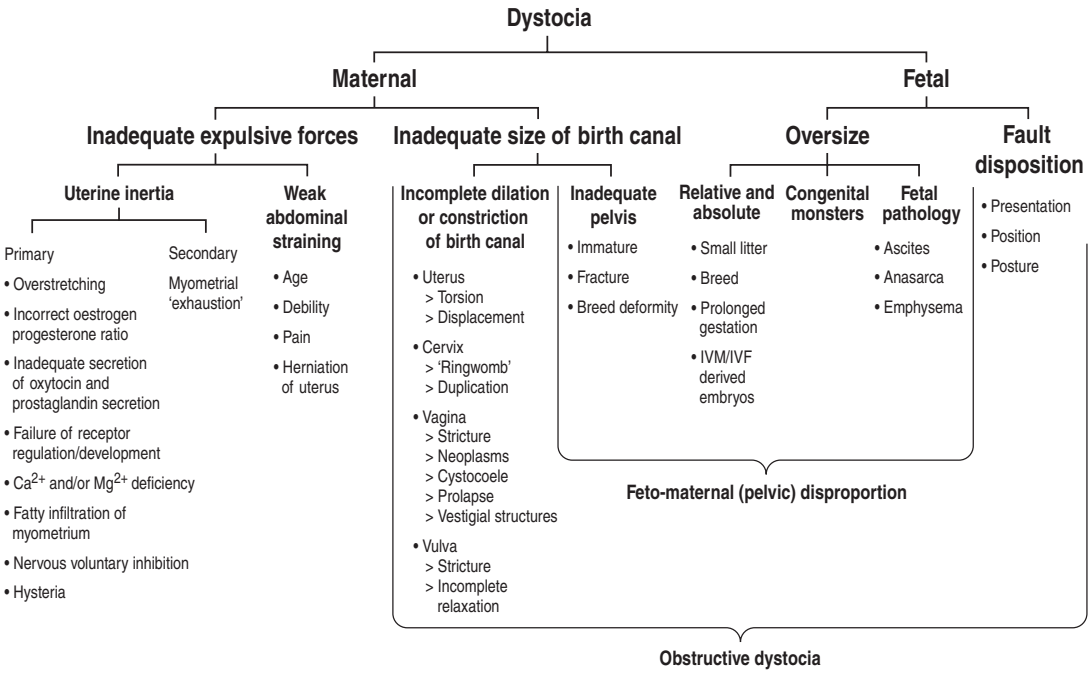

• **Fig. 11.1** The causes of dystocia.

Posture refers to the disposition of the movable appendages of the fetus and involves flexion or extension of the **neck or limb joints**, for example, lateral flexion of the neck or hock flexion posture.

Disproportion: Fetomaternal disproportion occurs when the fetus is larger than the capacity of the pelvis through which it has to pass. Disproportion may occur if the fetus is larger than normal, either in terms of its absolute size or of the size of particular parts of its body (notably shoulders and/or pelvis), or if the pelvic canal of the dam is too small or is of incorrect shape.

History and General Examination

History

Before examining the animal, an effort should be made to obtain a pertinent history of the case from the owner/attendant. Key points to be elicited from the history are summarized in Fig. 11.3.

Perhaps the most important aspects of the history are (i) the duration of labour and (ii) attempts that have already been made to assist the birth. The parity of the animal is the third key aspect because the incidence of different types of dystocia varies markedly with parity. For livestock, it is generally obvious whether the animal is primigravida or multigravida, but this can be rather less obvious in other species.

The duration of labour is important inasmuch as it is one of the key determinants of outcome for mother and fetus and often constrains the options available to the obstetrician for managing the case. Calculating the time the start of labour is often difficult because the signs of the onset of first stage labour are sufficiently vague and indistinct to easily escape the attention of an attendant. The onset of second stage labour, which is typified by vigorous and frequent straining, appearance of the amnion, expulsion of fetal fluids, appearance of a fetal extremity, and/or recumbency of the animal, are easier to recognize; this will typically be identified by an attendant as the 'start of labour'. Even so, in intensively managed livestock, it is by no means uncommon for the onset of even second stage labour to pass unnoticed and for the animal to be presented once a dystocia is obviously present, but with little indication of its duration.

As a generalization, it is reasonably certain that some form of obstructive dystocia exists if several hours have elapsed since the onset of second stage labour. The fetus can survive a moderate period of dystocia in most species, but this depends on the extent to which the fetus has traversed the birth canal before the labour is arrested. Thus, if the cause of the dystocia is relatively simple, such as slight fetomaternal disproportion or a minor limb malposture, and the case has not been neglected, fetal and maternal survival are often good. On the other hand, fetal survival of dystocia in the mare is short, as the normal course of delivery is so rapid and separation of the placenta occurs so quickly once second stage has commenced that any delay generally results in the death of the foal. If the dystocia has not been noticed until 24 or more hours after the start of second stage labour has started, the fetus is likely to have died and will often have started to become putrefied and decompose. Managing these cases is difficult because straining efforts have commonly ceased, fetal fluids have been entirely lost, and the uterus has started to contract down onto the retained fetus. In polytocous species, fetuses may survive for some time behind a point of obstruction, but eventually they will also die and/or start to decompose.

It is valuable to discover whether an attempt has already been made to assist the delivery. This may not only provide useful information about the type of dystocia that is (or is not) present, but may also provide evidence of whether injury has been caused either to the genital tract or to the musculoskeletal system of the animal. All of this information helps the obstetrician to provide a prognosis for mother and fetus for the outcome of the birth. It is particularly important to determine whether any drugs (commonly calcium, antibiotics, or oxytocin) have been given before assistance is requested, as this may affect the subsequent management of the case and will undoubtedly affect meat or milk withholding times for livestock.

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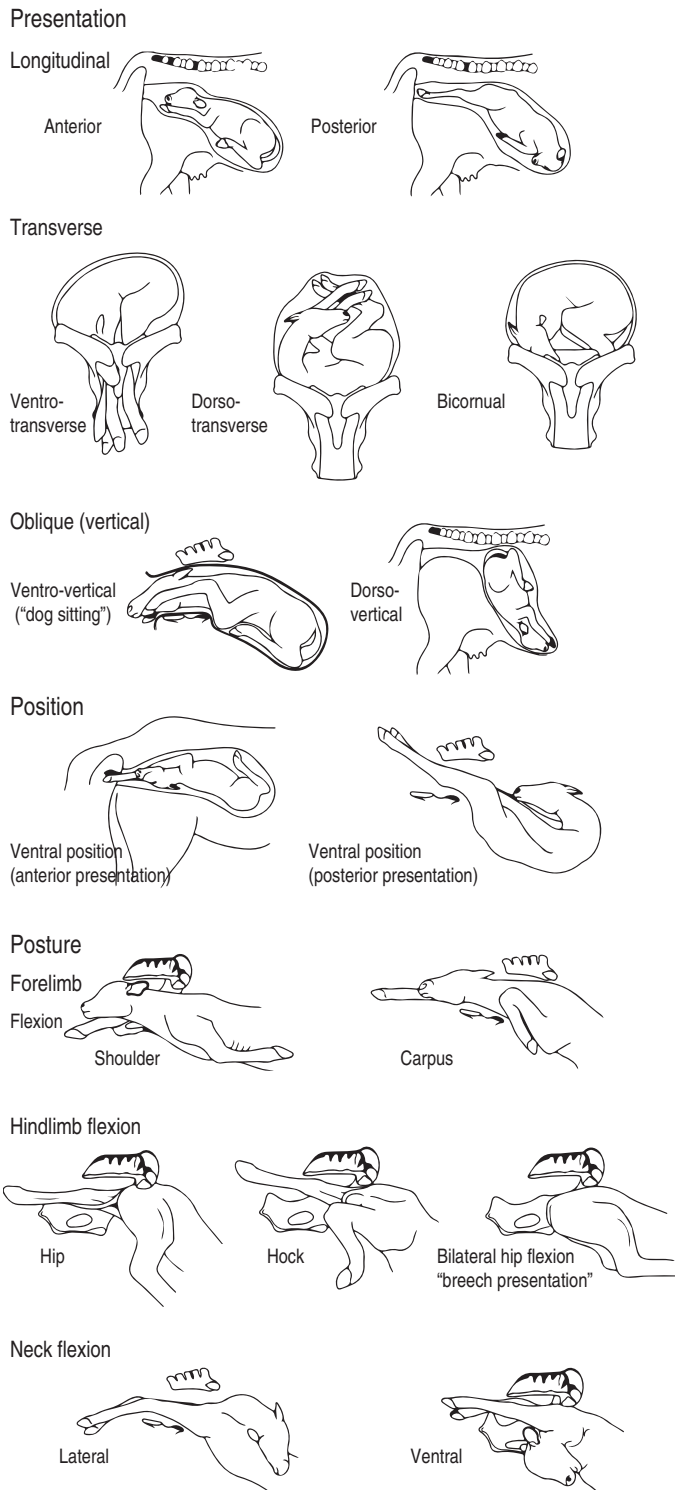

• **Fig. 11.2** Abnormalities of fetal presentation, position, and posture.

General Examination

The animal's physical and general condition should be noted. If recumbent, is she merely resting, is she exhausted, or is she suffering from a metabolic disease? Body temperature and pulse rate should be noted and the significance of abnormalities considered. Cattle should be routinely assessed for the presence of hypocalcaemia, and the possibility of toxic mastitis should not be overlooked.

The vulva should next be inspected. Parts of a fetus may be protruding, and it may be possible to assess the nature of the dystocia from these. Are exposed fetal parts moist or dry, or swollen emphysematous or oedematous? Such evidence serves not only as a guide to the duration of the condition but also to the effort that will be necessary to correct it. If parts of the amnion protrude, what is their condition? Are they moist and glistening with fluid caught up in their folds? If so, their exposure is recent, and the case is an early one. If, however, the membranes are dry and dark in colour, it may be taken that the case is protracted. Attention should also be paid to the nature of the discharge from the vulva, especially if neither fetus nor membranes are presented. Fresh blood, especially if profuse, generally indicates recent injury to the birth canal. A dark brown, fetid discharge indicates a grossly delayed case.

When dealing with the bitch and cat, the degree of abdominal distension should be observed, for it may be possible to make an estimate of the number of fetuses that occupy the uterus. The onset of vomiting, together with a great increase in thirst, should be regarded as grave signs in the bitch.

Detailed Examination of the Animal

Large Animal Species

The animal should be effectively restrained in a clean environment, for the safety of the veterinarian, any assistants, and the animal concerned. In the case of the mare (Fig. 11.4), cow, ewe, and doe goat, it is usually easier if they remain standing; the sow is best examined in lateral recumbency. One should never forget that large animals can collapse suddenly during examination, so it is important to ensure that (i) the animal can be easily extracted from the restraint if it does collapse and (ii) the obstetrician's hand/arm does not become trapped inside the animal when it collapses.

It may be necessary to sedate the animal before examining her – ideally, at a sufficiently early stage in the proceedings that she does not become fractious, but to a level that does not result in unplanned collapsing/recumbency. A decision whether to use epidural anaesthesia should likewise be made as early as possible. If the fetus is dead and/or the uterus is grossly contaminated, the epidural should be given before a vaginal examination is performed in order to minimize the risk of infecting the neural canal.

It is impossible to perform obstetrical procedures aseptically in any animal species, but the amount of contamination of the genital tract should be kept as low as possible. To this end, plentiful supplies of clean hot water with soap or surgical scrub should be available, as well as a table or bench covered with a sterile cloth on which instruments may be placed. Making sure that any bedding is clean (e.g., clean straw placed behind the animal) is also helpful. Because the floor is often wet and slippery, application of sand or grit is a worthwhile precaution.

The external genitalia and surrounding parts are thoroughly washed, and the tail is held or tied to one side. The obstetrician's hand and arms should be cleaned in water from a different bucket. The first vaginal examination usually provokes reflex defecation: more washing of animal and obstetrician is required. Some obstetricians prefer to wear a disposable plastic sleeve during examination of the animal: certainly this reduces the need for repeated rewashing of the obstetrician's arms, but some find it more difficult to differentiate tissues within the uterus when wearing a sleeve. When gross contamination of the uterus is present (e.g., in cases of bacterial abortions or when a putrefying fetus is present), it is very strongly advised that a sleeve should be worn. In the mare a

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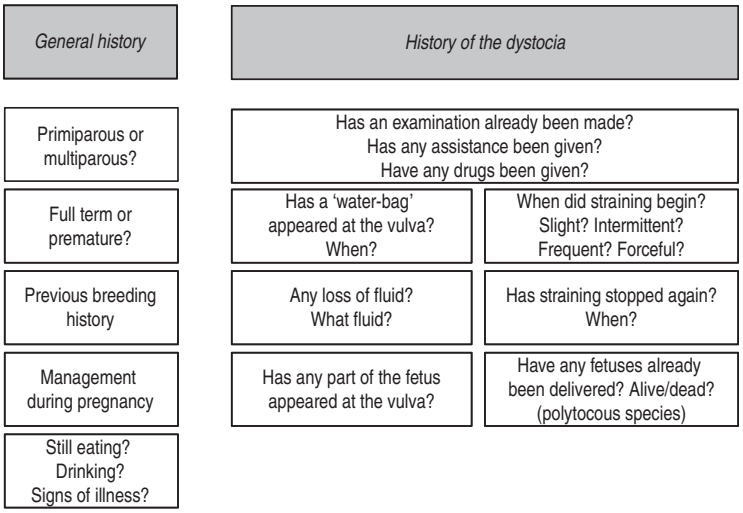
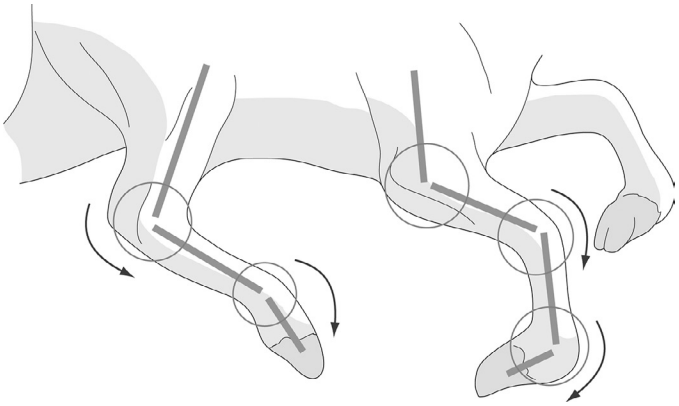


• **Fig. 11.3** Key points in the obstetrical history of a case.

• **Fig. 11.4** Mare with dystocia, restrained in stocks for vaginal examination and correction of dystocia.



• **Fig. 11.5** Determining whether presented limbs are fore- or hindlimbs. The olecranon and hock are easy to confuse during vaginal examination: however, in the forelimb, the first two movable joints (fetlock and carpus) flex in the same direction, whereas in the hindlimb, the first two movable joints (fetlock and hock) flex in the opposite directions.

clean tail bandage is applied because the tail hairs are frequently introduced into the vulva and vagina which can cause quite severe lacerations. Without the previous induction of epidural anaesthesia and the resultant paralysis of the rectum, it is almost impossible to make a vaginal examination in the cow without introducing some faecal contamination. There is little serious consequence of mild contamination, fortunately; but the greater the contamination that occurs, the greater the risk of postpartum uterine infection.

The next stage is to perform a vaginal examination in order to establish the patency of the birth canal, the orientation of the fetus, and the position of its movable parts (i.e., head, limbs, tail). Copious lubrication should be introduced to the birth canal at the start of the vaginal examination.

In the majority of cases, some part of the fetus occupies the vagina – the head, a limb, or limbs. Some difficulty can initially be experienced in recognizing the fetal parts being palpated if they are covered by amnion. To overcome this, the torn edges of the amniotic sac should be identified and opened, and the hand inserted so that the fingers come into direct contact with the fetus. It is often necessary to repel the fetus back into the uterus to ascertain the nature and direction of displaced parts. If continued straining makes this difficult, the induction of epidural anaesthesia should be considered at once, but it should be remembered that the dam's expulsive effort may be required after any corrective procedure has been performed.

Recognition of the head is not difficult; the mouth and tongue, the orbits, and the ears are generally obvious. In the case of limbs, the first requirement is to ascertain whether it is a forelimb or hindlimb. There are two main ways of assessing this: the orientation of the feet and the flexion of the joints of the limb. If the plantar aspect of the digit is downwards, it is most likely to be a forelimb; if the plantar aspect is upwards, it is most likely to be a hindlimb. This 'rule of thumb' nearly always holds true for the cow/ewe/doe, but in the mare, it is not uncommon for the fetus to be in the ventral position, so more caution is needed. The direction of flexion of the limb joints differs between forelimb and hindlimb: if the fetlock and the joint immediately above it flex in the same direction, it is a forelimb. Conversely, if the fetlock and joint above it flex in the opposite direction, it is a hindlimb (Fig. 11.5). If more than one limb is present, it must be established that they are either fore or hind and if they are from the same fetus.

If nothing is present in the vagina, the cervix should next be examined. First, it should be determined whether the cervix is completely dilated. If the cervix is largely, but not fully, dilated, a 'lip' of tissue will be evident, usually at the caudal end of the cervix. If less dilated, the cervical structure will be obvious, and there may even still be mucous within it. In the latter case the

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first thing to consider is whether the first stage of labour is not yet complete; i.e., the second stage of labour has not yet begun, and the animal requires more time. On the other hand, failure of cervical dilation can be a sign of dystocia. For example, the cervix usually fails to fully dilate in cases of uterine torsion, and failure of the fetus to impact the internal os of the cervix (i.e., through some form of faulty disposition) can also lead to partial failure of cervical dilation. Finally, it should be ascertained whether the cervix is incompletely dilated as the dystocia has been neglected, and the cervix has started to close again. This will usually be accompanied by complete loss of fetal fluids and often by obvious signs that the fetus has died and is starting to decompose.

When the fetus has not fully entered the birth canal, deeper palpation is needed to determine what parts are presented. For example, palpating the fetal tail and perineum means that there is a high likelihood that the fetus is in 'breech' presentation. Likewise, if a flexed neck is palpated and a search on one or other side reveals ears and occiput, the case is one of lateral deviation of the head. Similarly, presentation of the head but not of forelimbs can indicate flexion of the carpi or of the shoulders. In the mare (and sometimes in ruminants), it may indicate that the fetus is in a dorsotransverse presentation, or perhaps occupying both horns (bicornual gestation). Finally, the presentation of a plethora of limbs may indicate simultaneous presentation of twins, fetal abnormalities such as schistosoma reflexa or limb duplication, or a ventrotransverse or vertical presentation.

It may be worthwhile trying to assess the viability of the fetus, inasmuch as this can influence the options for management of the case. For cattle, however, it should be noted that, with increasing herd sizes and the associated improvements of the skills and knowledge of farm staff, it is increasingly unusual for the calf to be alive at the time when obstetrical assistance is requested. Viability can be assessed by attempting to elicit reflexes such as corneal/palpebral, suck, anal (if in posterior presentation), and limb withdrawal. Palpation for the presence of a pulse in the umbilicus may be feasible when the fetus is in posterior longitudinal presentation.

In the neglected or protracted case, assessment of the exact nature of the dystocia and methods of correction may be more difficult. The vaginal wall becomes progressively more oedematous, so that even the insertion of a hand and arm becomes difficult, and there is no room in which to carry out manipulations. Loss of fluid results in the dam's mucous membranes and fetal parts becoming dry. Contraction of the uterus directly on the irregular contour of the fetus makes retropulsion difficult or even impossible. In general, these cases have to be managed by relaxing the uterine muscle with a spasmolytic, such as clenbuterol, and/or by infusing replacement fluids into the uterus, and always by the use of large quantities of obstetrical lubricant. In such cases, it may be necessary to estimate the time interval since the fetus died. Fetal emphysema and detachment of hair means that the fetus has been dead for at least 24 to 48 hours. If there is no emphysema and the cornea is cloudy and grey, then it will have been dead for 6 to 12 hours.

Dog and Cat

The bitch, unless an exceptionally large one, should be placed standing on a table. It is preferable that a person with whom the animal is familiar should hold its head and be warned that even some quiet stoical bitches may resent a vaginal examination. Fetal numbers may be assessed in some bitches by gentle abdominal palpation or, more commonly, by the use of transabdominal ultrasonography. Ultrasonography has the added advantage of being able to determine whether the pups are alive by identifying the beating fetal heart. At a later stage in the examination, it might also be necessary to take radiographs.

As a general rule, the operator will proceed to make a digital examination *per vaginam,* especially in early cases in which it is likely that obstruction is the cause of the delay and also in protracted ones in which it is estimated that a single fetus only remains unborn. Nevertheless, cases will be met in which it is obvious that inertia has supervened, and there are several fetuses to be delivered, in which case immediate Caesarean surgery or hysterectomy is indicated.

Whether or not the hair is clipped from the area around the vulva before making a vaginal examination will depend on the length of the coat. In long-coated animals, it is a great convenience to do so; although it is impossible to render the area sterile, it should be thoroughly cleansed beforehand.

Sometimes on raising the tail it is seen that part of a fetus, a head or hind parts, is protruding from the vulva. Such a finding is more common in the cat than the bitch. The case is a simple one; traction on the exposed parts effects delivery without difficulty and, provided this assistance has been forthcoming early, it is probable that parturition will proceed normally. Occasionally, it is found that the vagina is occupied by a fetal head or buttocks which have become impacted. In the majority, however, the pelvic canal is unoccupied and obstruction occurs at the inlet.

The fetal disposition should then be determined. If the head is present, but the occiput and ears are palpated rather than the mouth, it is likely to be a case of vertex presentation. If a single limb is felt, but there is no sign of the head, the case is probably one of lateral deviation of the head. If the presentation is posterior, the tail may be recognized (although sometimes it is directed forwards over the fetal back). The hindlimbs may have entered the pelvis or they may be retained (flexed). The fetus may have rotated into ventral or lateral position.

Determination of fetal viability by attempting to elicit reflexes is unreliable.

Obstetrical Equipment

Manipulative Deliveries *per vaginam* in Large Animals

The most important obstetrical instruments are the clean and gentle hands and arms of the obstetrician.

There is, however, an array of equipment that can be used to assist in obstetrical procedures; arguably, the obstetrician should aim to possess a minimum of essential equipment and to be thoroughly conversant with the use of each item. For the veterinarian doing ambulatory visits to farms, studs, and other livestock units where parturient animals are kept, it is advisable to have a dedicated collection of **core obstetrical instruments** and other equipment that is always available in an emergency; in addition, a dedicated **Caesarean surgery kit** is also important (see Chapter 16 and, when working with cattle, a **fetotomy kit** (Chapter 15) should be carried.

Simple instruments that are easy to handle and convenient to sterilize are best. More complex equipment is occasionally required, and the important consideration is to know when the use of such complicated instruments is indicated. With the availability of better sedatives and anaesthetic agents and improved methods for fetotomy and Caesarean surgery, many of the long-established items of obstetrical instrumentation have become obsolete, and veterinarians have lost the skills to use them effectively. Despite this, many of

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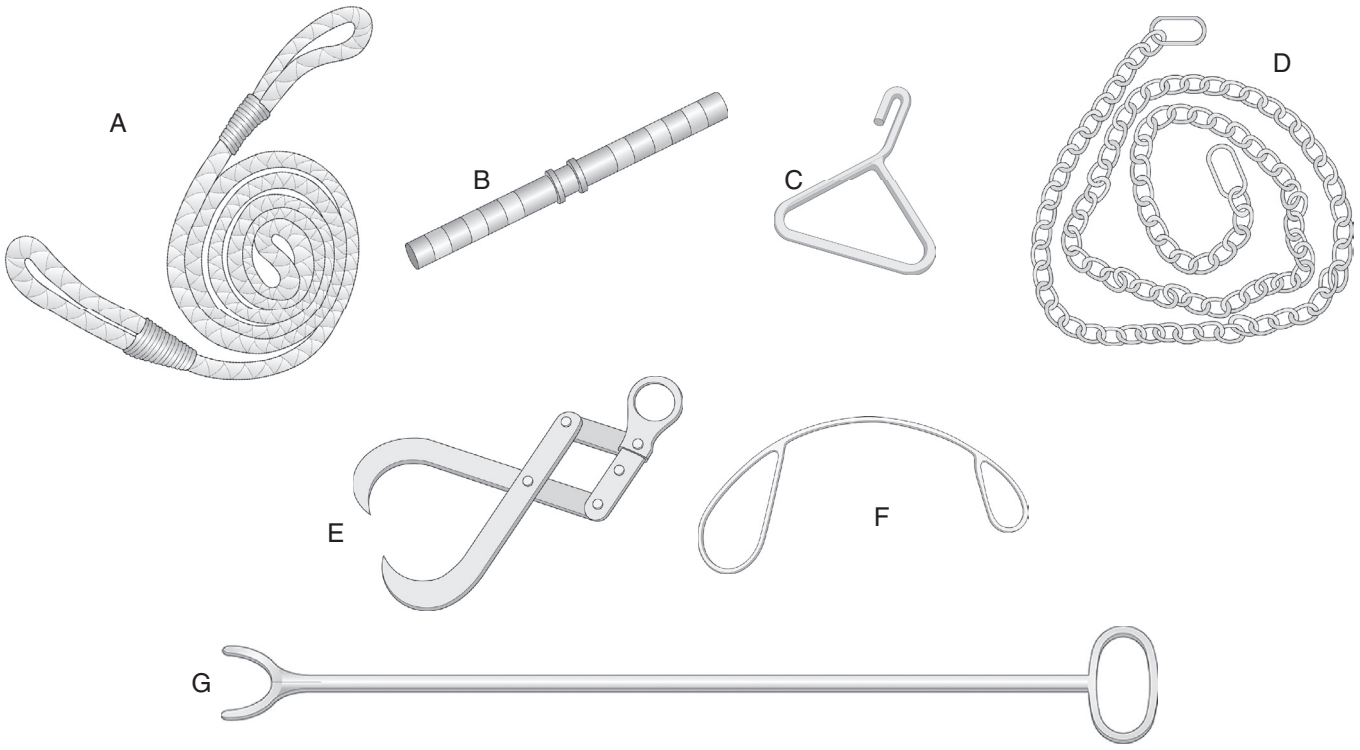

• **Fig. 11.6** Essential obstetrical equipment for use in cattle and horses. (A) Obstetrical snares made of cotton rope, nylon, or webbing. The snares have loops in both ends. (B) Traction bars for use with (A). (C) Obstetrical chain and (D) handles for applying traction. (E) Krey's hook (Krey-Schottler double jointed hooks). (F) Snare introducer for use with cord or chain snares. (G) Kühn's obstetrical crutch.

them can be very helpful at times and, for completeness, some of the more useful ones in cattle, and to a very much lesser extent in horses, are shown in Fig. 11.6. The most essential instruments are:

Obstetrical Snares

- Obstetrical snares are essential. Snares can be cord or chains.
- **Cord snares** are ~1 m lengths, with loops, of cotton rope (clothes line), nylon cord, or webbing (see Fig. 11.6A) (a finer cord for snaring the mandible is essential) and traction bars (see Fig. 11.6B). If cord snares are used, it is advisable to have at least two sets; they can be sterilized between uses.
- An alternative to snares that many veterinarians prefer to use is Moore's **obstetrical chains** (see Fig. 11.6D) with handles (see Fig. 11.6C). Many veterinarians find these easier to use than rope snares. Their main advantage is that they are heavier and do not move so readily when they are repositioned during intrauterine or intravaginal manipulation. It is also easier to clean them after use than cord snares.
- A **snare introducer** (see Fig. 11.6F) is also valuable for placing snares in tight spaces. It can be used with ropes as well as chains. A bull ring is an effective substitute

Traction

- A means of applying traction is also essential. Traction may be applied using a block and tackle or a calving aid such as an HK calf puller or Vink calving jack (Fig. 11.7).

Additional Equipment

- **Obstetrical hooks and crutches**. The main use of these is in fetotomy, and their use is described more fully in Chapter 14. Of these, probably the most useful is the Krey double-jointed hooks (see Fig. 11.6D). Less widely used are Obermeyer's anal hook, Harms's sharp or blunt paired hooks on a fine chain, and Blanchard's long, flexible cane hook. These are useful when performing fetotomy to enable traction to be applied to various fetal segments. Kühn's obstetrical crutch (see Fig. 11.6G) can also be useful for repelling the fetus in a tight space.
- **Detorsion rods and bar** are valuable aids to managing uterine torsion in the cow, and many veterinarians in cattle practice choose to have these as a part of their core kit.

Equipment for manipulative delivery of small animal fetuses is described in Chapter 18.

Specialised Equipment

Fetotomy is arguably the best alternative to the Caesarean surgery for delivering a dead, decaying, or emphysematous calf, so carrying a fetotomy kit is recommended. The kit includes a wire saw fetotome, cutting wire, handles and wire cutters, a fetotome threader, an introducer for positioning the cutting wire, and a set of Krey's hooks.

General Equipment

A general kit should be readily at hand alongside the obstetrical equipment. This consists of the following:

- Casting rope, halter, a 'general purpose' rope
- Buckets for washing water, antiseptic detergent
- A source of light (e.g., powerful torch and spare batteries)
- Core drugs: antibiotic, oxytocin, clenbuterol, local anaesthetic, analgesic (i.e., NSAID), injectable calcium (for cattle), sedatives; plus needles and syringes that are appropriate for the drug and its route of administration

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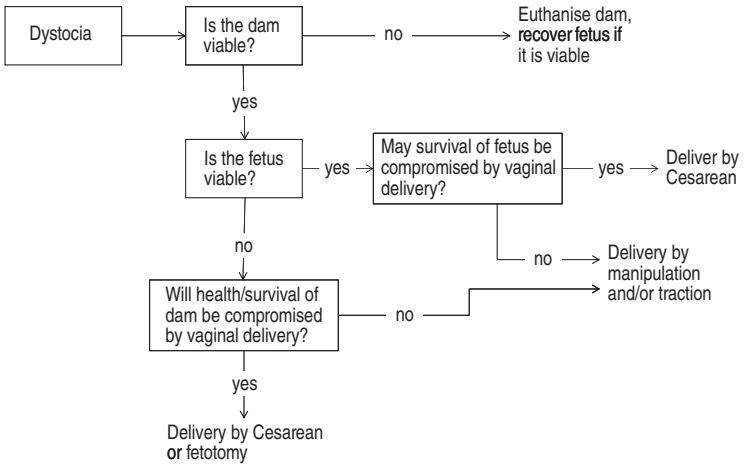
• **Fig. 11.7** Calving aids: (A) Vink calving jack, (B) HK calf puller. (Photo courtesy J. Malmo.)



• **Fig. 11.8** Decision pathway for managing dystocia.

Consideration of Treatment

General

The ideal outcome for any obstetrical procedure is the delivery of live and viable young without compromising the health or future fertility of the dam. When both fetus and dam are alive at the start of the procedure, this is indeed the primary measure of success. Nonetheless, there are situations in which the fetus is not viable or is dead at the start of the procedure. There are also situations in which the fetus is not viable, but the way in which that fetus is delivered will have a significant effect upon whether or not the dam survives (and/or is healthy and fertile). Finally, there are occasions when neither the fetus nor the dam are viable, in which case euthanasia may be the most humane and most cost effective way of resolving the situation. Hence a cascade of decisions has to be made (Fig. 11.8). If neither dam nor fetus will survive, euthanasia is appropriate. If the dam may survive, but the fetus will not, a decision regarding the most appropriate obstetrical method to extract the fetus while minimizing the risk to the dam has to be made. Rarely, the fetus may be viable but the dam not, in which case euthanasia of the dam/post mortem Caesarean may be a way of salvaging the fetus. Finally, if both dam and fetus are viable, different decisions have to be made about the best way of separating them.

No less important than this decision process is an assessment of the duration of the dystocia and the attempts that have been made to deliver the fetus. For example, a carpal flexion is a very simple dystocia to correct if the case is presented soon after the onset of second stage labour. If the dystocia has been neglected for a significant period of time, the fetal fluids will have been lost, the uterus may be contracting down on the fetus, and the fetus may be severely impacted in the birth canal. In such circumstances, manipulative correction of even such a simple faulty posture may not be feasible. If the dystocia has been neglected for even longer, the

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fetus may have started to decompose and become emphysematous, so that vaginal delivery of the intact fetus is no longer possible.

In cattle the duration and degree of traction to which the cow has been subjected is a major determinant of the outcome of subsequent Caesarean surgery. Similarly, in horses the duration of dystocia and the degree of trauma to the uterus significantly affect the outcome of subsequent interventions. However, with the advent of new and safer anaesthetic agents, Caesarean surgery should not be considered as 'the last resort' but as an effective method of treatment when used appropriately. Moreover, fetotomy should not be disregarded as a method of treating dystocia: in cattle, it probably remains the treatment of choice for grossly neglected cases in which the calf is decomposing and emphysematous or if the uterus is grossly contaminated.

Taken together, therefore, there should be an early commitment in the management of an obstetrical case as to whether it should be managed by manipulation plus or minus traction, by fetotomy, or by Caesarean surgery. As a general rule, failure to make progress by manipulation and/or traction within a reasonable period of time means that the original decision about the management of the case should be review, and the decision to proceed to an alternative method (Caesarean, fetotomy) should be made before either the veterinarian or patient become exhausted. The adverse consequence of traction that is either excessive in force or duration (such as laceration and contusion of maternal soft tissues, perineal laceration, rectovaginal fistula, pelvic nerve damage, hindlimb paralysis, or sacroiliac dislocation) are so well-known that there is no merit in persisting with traction beyond a point when it has ceased to result in progress towards the delivery of the fetus.

Of course, veterinarians will sometimes be pressurized by owners into performing Caesarean surgery when it is not necessary, particularly in cows with muscular hypertrophic calves or brachycephalic/achondroplastic bitches, purely because owners want to ensure the birth of live offspring. Likewise, owners will sometimes request the use of severe and prolonged traction rather than pay for the cost of Caesarean surgery. An ethical dilemma can then ensue as the veterinarian tries to balance the welfare of the patient with the expectations of its owner.

Horse

The first consideration is whether attempts at correction should be made with the animal standing or recumbent, or restrained and sedated, or under caudal epidural or general anaesthesia. The decision will be influenced in part by the size and temperament of the mare, by the type of dystocia, by the personnel to assist with the delivery, and by the accessibility of faculties for general anaesthesia and surgery (Miller 2010). Not infrequently, the operator begins manipulative correction with the mare unsedated and standing but soon realizes that, for success, restraint and/or sedation will be required. It is important in such cases that this decision be made early so that neither obstetrician nor mare becomes exhausted as the result of prolonged, futile efforts. In all severe cases the operator should consider the advisability of seeking the assistance of a colleague, for it is always possible that the combined efforts of two will succeed where those of one alone fail.

Relatively simple abnormalities, such as carpal flexion or lateral or downwards deviation of the head, can often be corrected using the hand alone, particularly when the mare is comparatively small and straining has been eliminated. However, it must be remembered that the limbs of the thoroughbred newborn foal are very long (70% of their adult length), which requires a substantial amount of space to facilitate flexion and extension. When, however, one of the more difficult forms is present, such as transverse presentation, ventral position, or impaction of the fetus in the pelvis, or when there is laceration of the vagina or vulva, it is generally best to anaesthetize the animal at the outset, particularly in a hospital environment. One of the advantages of general anaesthesia is that by changing the position of the mare – for example, so that she is in dorsal or lateral recumbency or even suspended by her hindlimbs (the anaesthetists will not be very enthusiastic about this approach because of pressure on the diaphragm!) – the change in the pressures on the fetus within the uterus can be utilised to facilitate correction. Whenever fetotomy is required, both sedation and caudal epidural anaesthesia should be used. In veterinary hospitals, general anaesthesia is preferable because the foal is almost always already dead and will not be affected by transplacental transfer of anaesthetic agents.

The value of partial fetotomy as a treatment of equine dystocia in which the fetus is dead or deformed has long been recognized. Vandeplassche (1980) considered partial fetotomy to be the method of choice for rapid, safe correction of dystocia that cannot be resolved by manipulation. However, Frazer (2001) noted that fetotomy should be avoided when the reproductive tract of the mare is already traumatized by failed attempts at manual correction. Carluccio et al. (2007) described that deliveries could commonly be made with just two cuts. The indications for partial fetotomy are shown in Table 11.1. Sequelae of fetotomy have remained surprisingly consistent over many years: the survival rate is between 85% and 90%, around 25% to 35% of mares have retained fetal membranes and/or postpartum endometritis, and fertility in the first season after fetotomy is around 30% to 40% (Vandeplassche 1980, Ras et al. 2014).

With improved methods of general anaesthesia and aseptic surgery, Caesarean surgery has a definite place in equine obstetrics, particular indications being maternal dystocia due to bicornual gestation, uterine torsion, and narrow or deformed pelvis, as well as those cases of fetal dystocia in which there is oversize or faulty fetal disposition combined with maternal injury or when the uterus has contracted on to a dead, emphysematous foal. In the series of Caesarean cases reported by Abernathy-Young et al. (2012), 75% were due to dystocia, with most of the remainder due to concurrent maternal disease (i.e., colic) that compromised the fetus. Interestingly, 16% of the Caesarean were performed after a failure of partial fetotomy. Survival rates have not changed much over the years, however: Vandeplassche (1980) recorded 81% survival whereas the figure achieved by Abernathy-Young et al. (2012) was 84%. Hodder et al. (2012) noted that survival of emergency Caesareans was lower than that for elective surgery (75% vs 100%). Foal survival is poor for emergency Caesareans, as most foals were dead or severely compromised before the onset of surgery.

Cattle

The most common cause of dystocia in cattle is fetomaternal disproportion, with faulty disposition of the fetus being the next most common in both beef and dairy cattle (Meijering 1984, Citek et al. 2011). In principle, these dystocias can be delivered by correction of the fetal disposition or delivery by traction. However, the most important factor in determining the course of action in

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TABLE 11.1 Prevalence of different forms of dystocia in mares undergoing fetotomy

Cause of Dystocia	Vandeplassche	Carluccio	Total	(%)
Anterior presentation: lateral or ventral deviation of the head	72	31	103	(50.5%)
Anterior presentation: hydrocephalus or two heads	6	1	7	(3.4%)
Anterior presentation: flexion, deformity, or ankylosis of forelegs	12	18	30	(14.7%)
Breech presentation with ankylosis	17	5	22	(10.8%)
Posterior presentation, hock flexion		8	8	(3.9%)
Transverse presentation	25	5	30	(14.7%)
'Dog-sitting' position		1	1	(0.5%)
Others		3	3	(1.5%)
Total	132	72	204	

Derived from Vandeplassche (1972, 1980) and Carluccio et al. (2007).

bovine dystocias is not (usually) the cause of the dystocia, but the interval that has elapsed between its onset and the presentation of the case for examination.

In neglected or protracted cases, there is often severe impaction of parts of the fetus in the pelvis; the greater part of the fetal fluids has often been lost, and there is insufficient space to repel the fetus; the fetal skin and the vaginal mucosa have lost their natural lubrication, whereas the vagina and vulva are often swollen, and manipulation is rendered difficult. Correction of the dystocia, whether due to faulty disposition or fetomaternal disproportion, in such cases may prove very difficult and may necessitate an early decision to undertake fetotomy or Caesarean surgery in preference to attempting vaginal delivery.

Fetomaternal disproportion is more common in heifers than in multigravida cows (Zaborski et al. 2009). Traction is the first method of delivery that should be attempted in most cases of fetomaternal disproportion. Before traction is applied, the vagina and those parts of the fetus occupying it must be thoroughly lubricated with copious application of several litres of a proprietary cellulose-based obstetrical lubricant. In cases in which the fetal disposition is normal and the disproportion is slight, it is a comparatively simple matter to apply snares to the fetal extremities (i.e., the limbs and head) and, adhering to the principles described in Chapter 14, the fetus is delivered by traction. As a rule, the animal remains standing during the application of snares but often goes down during the passage of the calf's head through the vulva. More severe disproportion requires more careful assessment and, as described previously, an early decision to use an alternative to traction when this is unlikely to be successful. Traction, however, must be employed with care because excessive traction can not only cause damage to the dam, but can also cause the fetus to become so tightly impacted in the birth canal that its subsequent delivery by Caesarean surgery or fetotomy can become difficult or impossible. Hence the amount of traction must be limited to that of three persons or a calving aid and the progress of the operation must be very closely scrutinized by the veterinarian. If no progress is made after 5 minutes or if the fetus becomes lodged and fails to yield to 5 minutes of further traction, then partial or total fetotomy or Caesarean surgery should be performed (Chapters 15 and 16).

Faulty fetal disposition is more common in parous cows than in primigravida. When faulty disposition is the cause of the dystocia, an assessment needs to be made of the measures that will be needed to correct it. Sometimes the space required for correction is lost due to the effects of continual straining or the contracted uterus. If so, one or all of caudal epidural anaesthesia, clenbuterol, and/or infusion of replacement fetal fluids should be given without delay. Occasionally, inducing epidural anaesthesia will allow an animal that has become recumbent to rise, which makes any manipulative procedures easier because the veterinarian can stand and the intraabdominal pressure is reduced.

Management of dystocia due to fetal 'monsters' again requires an early assessment of the best means of delivering the calf. Many 'fetal monsters' cannot be delivered intact *per vaginam* and will require either fetotomy (usually) or Caesarean surgery (sometimes). It should, however, be noted that for fetal monsters for which Caesarean surgery is the method of choice (e.g., schistosoma reflexus in which the head and limbs are directed towards the pelvic inlet), fetotomy is contraindicated.

Sheep and Goat

The ability of a veterinarian to resolve dystocia in the ewe or doe largely depends on the operator's ability to pass a hand through the pelvis into the uterus. Primigravid animals of the smaller breeds sometimes foil attempts of even the smallest hand to traverse their birth canal, making delivery by Caesarean surgery the best option for success.

Cases of fetomaternal disproportion are relatively uncommon, except in ewes carrying a singleton fetus. Most cases have normal fetal disposition, so once the head has been repelled from the pelvic inlet, gentle traction upon the limbs effects delivery. Use of a lambing snare (Fig. 11.9) to control the direction of the head is often helpful in cases of fetomaternal disproportion. When there is malposture of the limbs or head, repositioning after retropulsion is, as a rule, relatively easy. Retropulsion, replacement of lost fetal fluids, and correction of a faulty disposition are made much easier by, first, giving causal epidural anaesthesia and, second, elevating the hindquarters of the ewe. This can be done by rolling her on to her back and having an assistant pull both hind legs upwards and

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• **Fig. 11.9** Lambing snare.

forwards. It is especially important to ensure that the presented parts all belong to the same fetus. The young, in cases of twins and triplets, are small and retropulsion and reposition are seldom difficult. Gentleness is critical in handling dystocias of the ewe and doe, as it extremely easy to damage the birth canal, pelvis or obturator nerve. Likewise, great care must be taken during intravaginal manipulations that the mucous membrane at the pelvic inlet is not lacerated. It is an accident that may occur quite simply, particularly when a finger is being used to lever a head or limb upwards. Such lacerations are usually followed by infection and possibly death.

In most cases in which manipulation and delivery *per vaginam* is not possible, delivery by Caesarean surgery is usually the best option.

Pig

In the sow, the ease with which obstructive dystocia can be relieved depends almost entirely on the operator's ability to pass a hand through the pelvic inlet. Provided this is possible, it is usually a relatively easy matter to grip the head or hind parts and withdraw the fetus. In small gilts and in sows of breeds such as the Vietnamese pot-bellied breed, the use of a lambing snare (see Fig. 11.9) may be useful to apply traction to the head. The disposition of the limbs is seldom of much consequence. When assistance is given early in the dystocia (i.e., within an hour or two of the onset of second stage labour), removal of the obstructing fetus is often followed by the normal expulsion of the remainder. Assistance in the sow is frequently delayed, however; in these cases the obstetrician will be well advised to remove as many piglets from the uterine body and cornua as are within reach. The subsequent course will depend chiefly on the measure of delay and thus the degree of inertia which has supervened. It may be found in an hour or so that normal expulsion has recommenced or that on further examination more fetuses are accessible to manual extraction, and by continued attention to the sow in this manner the whole litter can be removed. It is worth remembering that intravaginal and intrauterine manipulations will stimulate the release of endogenous oxytocin and thus stimulate myometrial contractions. Quite often, however, complete inertia has developed and no further progress follows the removal of the accessible fetuses. In these, Caesarean surgery is the only means of saving the sow.

The strategic use of oxytocin to induce myometrial contractions can be used to treat overt cases of dystocia and also to hasten the expulsion of piglets if there is an extended time interval before the arrival of the next, thus preventing stillbirth. It is important to give low doses of oxytocin because it is a potent ecbolic, and large dose rates will cause spasm of the myometrium rather than rhythmical peristaltic-like contractions. In addition, the myometrium will become refractory to its effect, and it may be necessary to increase the dose rate in order to obtain a response. The dose of oxytocin is therefore critical to its successful use in farrowing. Mota-Rojas et al. (2006) noted that too high of a dose (or too rapid elevation of blood concentrations, particularly by IV administration) was associated with higher rates of fetal asphyxia/fetal distress, but that a low dose of oxytocin (0.083 IU/kg) gave a useful acceleration of parturition without causing fetal distress (Mota-Rojas et al. (2005) and improved survival of piglets experiencing dystocia (González-Lozano et al. 2009). Jackson (1996) observed that the greatest problem in porcine obstetrics was to know when a parturient sow had expelled all her piglets. Good, but not infallible, indications of the end of labour are that the sow rises, passes a large volume of urine, and then resumes recumbency in an attitude of contentment.

When it is suspected that parturition is incomplete, the clinician should pass a hand as far as possible into the uterus and sweep it gently about the abdomen in the hope of balloting indirectly a piglet in an adjacent segment of the long uterine horn. Transabdominal ultrasonography can potentially be used to locate a retained fetus (see Chapter 17). The presence of retained fetal membranes is even more difficult to determine. When the clinical manifestations suggest that a fetus (or fetuses) is still retained and there has been no response to the administration of ecbolics, the only approach would be an exploratory laparotomy. Sows and gilts will often survive the presence of retained piglets, which sometimes become mummified (see Chapter 17). Because they are occasionally seen in the uteri of culled sows and gilts at the abattoir, it is likely that, although the mothers survived, they were infertile.

Dog and Cat

The primary consideration in the management of a case of dystocia in the bitch or queen is whether one should attempt delivery *per vaginam* or immediately resort to Caesarean surgery. Factors that will influence the decision are:

- The cause of dystocia, whether obstruction or primary inertia;
- The duration of second stage labour and hence the condition of the fetuses and the uterine muscle; and
- The number of fetuses retained and their viability.

When the case is recent (i.e., a few hours only), attempting vaginal delivery may be appropriate. A modest degree of fetomaternal disproportion with the fetus in anterior or posterior presentation may be resolved by traction using the fingers only (see Figs 18.1 and 18.4), or finger and vectis, or forceps (these should be used with great care to prevent trauma to both dam and offspring); this traction will succeed in effecting delivery, and parturition will then proceed normally. Similarly, in cases of faulty fetal disposition, such as vertex posture or breech, traction may succeed after correction of the abnormal posture.

Increasingly commonly, however, the decision is likely to proceed directly to Caesarean surgery. Certainly, in cases of gross fetomaternal disproportion (which is very common with litters of

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one or two) or in cases in which the dystocia is of more than a few hours duration, Caesarean surgery is indicated. The obstetrical justification for this decision is that it is very likely that secondary inertia has supervened and removal of the obstructed fetus will not alter the ultimate outcome. This decision is often, however, subordinate to an economic justification: Caesarean surgery is used to minimize the risk of death of the dam and litter of pups or kittens – both of which may be valuable. The question sometimes arises of whether one should first attempt to remove the presented fetus *per vaginam* before performing surgery. It is highly likely that this fetus is contaminated, and withdrawing it through an abdominal wound may predispose to development of peritonitis. There is also, of course, the possibility that forceps interference will subject the bitch or queen to greater risk. Many veterinarians are of the view that the risk to the dam and the rest of the litter is minimized if the presented fetus is removed during the Caesarean surgery unless there is a very clear indication indeed for its removal *per vaginam.*

The length of time for which the fetus will remain alive after the onset of second stage labour also has to be considered. It is very improbable that the presented fetus will live longer than 6 to 8 hours, for by that time its placenta will have completely separated. The remaining fetuses, however, may be alive for much longer periods. Thus it is possible that after 36 hours' delay, the presented fetus may be dead with early signs of emphysema, yet those occupying the anterior parts of the cornua may be alive. After a delay of 48 hours, this is highly unlikely to occur.

Hysterectomy may be chosen as an alternative to Caesarean surgery (see Chapter 19). Robbins and Mullen (1994) showed that newborn survival rates of 75% for dogs and 42% for cats are comparable to the outcomes of Caesarean surgery to treat dystocia.

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12

Prevalence, Causes and Consequences of Dystocia

TIMOTHY J. PARKINSON, JOS J. VERMUNT AND DAVID E. NOAKES

Introduction

The diagnosis and treatment of dystocia requires a good understanding of normal parturition, sensitivity to the welfare of both dam and offspring, and good practical competences.

Developing an adequate definition of dystocia that can be applied to scientific study of the causes, efficacy of treatment, and consequences of dystocia has not proved to be an easy task. Although a simple binary classification into 'dystocia' (i.e., a difficult birth) and 'eutocia' (i.e., a normal birth) may seem straightforward, the diagnosis of dystocia is actually highly subjective, inasmuch as situations that one person will consider to be normal will be considered as difficult by another. Attempts have been made to increase the objectivity of the diagnosis of dystocia by, for example, at one end of the spectrum creating a hierarchical classification of dystocia according to the level of intervention that is required (Dematawewa & Berger 1997) or, at the other, through a binary classification into 'assisted' or 'not assisted' births (Morton 2000).

In principle, dystocia occurs when there is a failure of either the expulsive forces or the adequacy of the birth canal, or the disposition of the fetus. These failures relate, ultimately, to genetic factors derived from the sire and dam and management factors relating to the dam during and before pregnancy. Taking this concept further, Mee et al. (2011) identified proximal ('immediate'), intermediate, and ultimate causes of bovine dystocia. Proximal causes are those problems that one identifies at the time of an obstetrical intervention – such as fetomaternal disproportion, fetal maldisposition, uterine inertia, or uterine torsion (Fig. 12.1). In the example of fetomaternal disproportion, intermediate causes are calf birth weight and maternal pelvic size. The ultimate causes for these intermediate factors include the gender and breed of sire of the calf, as well as the parity, weight, age, and condition score of the dam. In fact, fetomaternal disproportion very nicely illustrates the interplay of factors that cause dystocia, as it is neither strictly fetal in origin nor is it strictly maternal. Sometimes it is indeed due to an absolute 'oversize' of the fetus, but it can equally be due to an absolute 'undersize' of the birth canal or pelvis of the dam, or, in most cases, the fetus is relatively too large and the dam relatively too small so these two are disproportioned to each other. Regardless, the condition represents a complex interplay between sire, dam, and environmental factors that can need to be carefully teased apart to understand the ultimate cause of the dystocia. Only when the ultimate causes have been identified can effective preventative measures be instituted.

Prevalence

Cattle

Obtaining a generalised figure for the prevalence of dystocia is beset by the difficulties of classification of the severity of dystocia that have been mentioned. Adopting the convention that a 'hard pull', surgery/fetotomy, or stillbirth is a 'dystocia', whereas lesser interventions are an 'assisted calving' (Lopez Helguera et al. 2016), an overall figure of 4% to 6% of births appears to be remarkably consistent across time, farming systems, and country. Thus Mee (2008) reviewed recent literature that showed the overall prevalence of dystocia in dairy cattle to range from 1–5% to 6.6%, although the proportion of calvings that were assisted was very much higher (varying between 10% and 50%) (Hansen et al. 2004, Heringstad et al. 2007). The risk of dystocia is consistently higher in primiparous than multiparous cows. Edwards (1979) found that dystocia was more common in heifers than multiparous cows, such that there were 66.5%, 23.1%, and 14.3% assisted deliveries in first, second, and third calvings respectively. Many subsequent studies have confirmed this trend (Sieber et al. 1989, McDermott et al. 1992, Mee et al. 2011). Citek et al. (2011) reported a prevalence of 4.1% across a range of beef breeds, and McDermott et al. (1992) reported that of the 8.7% of beef breed calvings that required assistance, only 3.4% were regarded as 'difficult'. Some breeds are more at risk of dystocia than others: large, or 'double muscled' beef breeds are at greater risk than other beef breeds. Among dairy breeds, the prevalence of dystocia in Holstein heifers is worryingly high, with figures of 17%, 21%, 22%, and 40% of all primiparous births requiring assistance (Meyer et al. 2001, Mee 2008, Mee et al. 2011). Lopez-Helguera et al. (2016) similarly recorded a figure of 40% Holstein–Friesian heifers requiring assistance, 9% of which were also classed as having dystocia.

Fetomaternal disproportion is the most common overall cause of dystocia in cattle, with a relative frequency of about 50% of all dystocias (3.5% of all births). It is much more common in primiparous than multiparous animals, whereas dystocia due to faulty disposition of the fetus is relatively more common in multiparous than primiparous animals (Meijering 1984). Some studies have attempted to provide separate prevalences for the 'maternal' and the 'fetal' components of fetomaternal disproportion. For example, Citek et al. (2011) reported that 53% of dystocias in beef cattle was due to fetomaternal disproportion, of which 24.5% was due to fetal oversize and 28.5% due to narrowness of the maternal pelvis.

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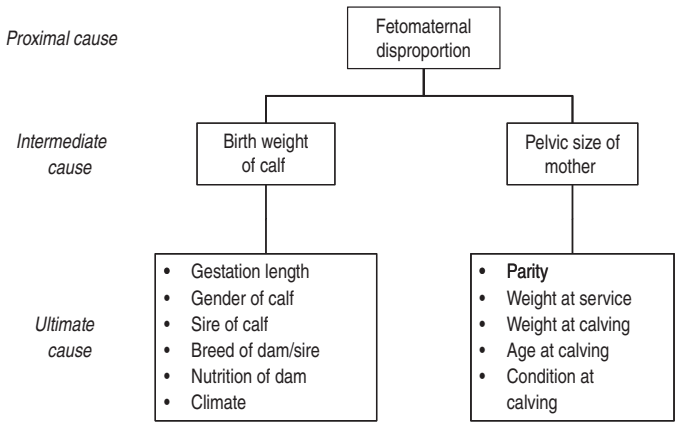
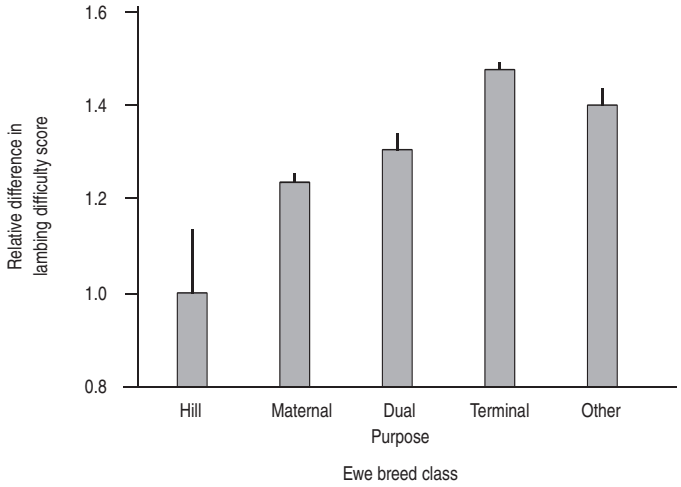


• **Fig. 12.1** Intermediate and ultimate causes of dystocia due to fetopelvic disproportion in dairy cattle. (Based upon Mee 2008.)



• **Fig. 12.2** Mean (SE) lambing difficulty score for ewe breed class relative to the hill ewe breed. (Derived from McHugh et al. 2016.)

Dystocia due to faulty disposition of the fetus is less common, variously estimated at 17% to 30% of dystocias (2%–4% of all births). Citek et al. (2011) estimated that faulty posture, position, and presentation accounted for 7.8%, 2.3%, and 8.2% of dystocias, respectively. Many of these dystocias due to 'faulty' disposition are, in fact, uncomplicated posterior presentations, which occur in about 5% of all births and which results in dystocia in up to 50% of such births. Departures from longitudinal presentation are uncommon because the anatomical arrangement of the uterine cornua and the absence of a distinct uterine body in the cow do not favour transverse presentation. Postural irregularities of the head and limbs are relatively common, particularly carpal flexion, lateral deviation of the head, and 'breech presentation'. Thus Holland et al. (1993), who recorded the prevalence of faulty disposition of the fetus over a 21-year period in Colorado, United States, found that abnormal disposition of the fetus occurred in 4% of calvings. Of these, 73% were uncomplicated posterior presentations, 8% were in breech position, and 1% was in posterior longitudinal presentation and ventral position. Of those in anterior presentation, 11% had unilateral carpal or shoulder flexion, 2% had incomplete extension of the elbow, and 2.5% had lateral deviation of the head. Transverse presentation and oblique ventrovertical presentation/position were present in 1.4% and 0.6% of cases, respectively.

Twin calvings, which occur in 2.5% of births, have about a 2.5 times greater risk of dystocia than do singleton births, although Correa et al. (1993) estimated that, in Holstein cows, the risk of dystocia in multiple births was 10 times higher than in singleton births. Simultaneous presentation of twins is a well-recognised cause of bovine dystocia, and one of the first duties of the obstetrician when proceeding to manipulative delivery is to ensure that the presenting limbs belong to the same fetus.

The prevalence of dystocia due to anatomical defects of the dam is relatively low: the prevalence of uterine torsion has been estimated at around 7% to 10% of dystocias requiring veterinary attention (0.25% of all births) and incomplete cervical dilation at around 2% to 5% of cases requiring veterinary attention (see Ben-David [1961], Meijering [1984], Colnar et al. [2008], Mee et al. [2011], Purohit et al. [2011], Lyons et al. [2013], Uematsu et al. [2013]). Uterine inertia, often associated with hypocalcaemia, is well known, particularly in pluriparous cows (>5 years of age).

The prevalence of fetal monsters is relatively high in the cow. They are generally of the distorted and celosomian types, schistosoma reflexus and perosomus elumbis being the most common (see Chapter 9). Knight (1996) reported a prevalence of 1.3% of the dystocias attended by veterinarians in Victoria, Australia, were due to schistosoma reflexus. Likewise, in a survey of calvings in Poland from 1970 to 1974, 13% of fetuses or newborn calves with developmental congenital abnormalities were also due to this abnormality (Cawlikowski 1993).

Sheep and Goats

Ewes. The prevalence of dystocia in sheep is influenced by breed, parity, and litter size. Estimates of prevalence vary greatly between breed, such that hill breeds tend to have lower dystocia scores than other breeds, particularly terminal breeds (Fig. 12.2). Among UK hill sheep, dystocia rates reported for the Scottish Blackface, Cheviots, and Welsh Mountain breeds vary between 1% and 6% (Gunn 1968, Whitelaw & Watchorn 1975, Woolliams et al. 1983, Speijers et al. 2010), whereas among the terminal breeds, rates of 34% (Dorset Horn), 31% (Suffolk), and 56% (Texel) have been reported (George 1976, Grommers 1977, Dwyer & Bunger 2012). It is, however, important to recognise that variation in the definition of dystocia between studies makes it exceedingly difficult to make comparisons between trials of absolute rates for dystocia in different breeds.

Fetomaternal disproportion and faulty disposition of the fetus are the two most common causes of dystocia in sheep (Fig. 12.3). Fetomaternal disproportion is related to particular breeds, and the risk for development of the condition is high when there is crossing of disparate breeds for commercial lamb production. Although lamb birth weight is an important contributor to fetomaternal disproportion, such that the risk of dystocia rises markedly >6 kg birth weight, *lamb mortality* increases when birth weight falls <3 to 4 kg (Refshauge et al. 2007, Speijers et al. 2010).

The relative risk of faulty fetal disposition as a cause of dystocia is higher in ewes than in cows but, although the relative proportions of disproportion *versus* faulty disposition varies among studies, this appears to be largely unrelated to breed. Maldisposition of the fetus is more common with multiple than singleton births (Gunn 1968). Pluriparous ewes are relatively more likely to experience dystocia due to faulty fetal disposition than are primiparous animals, and

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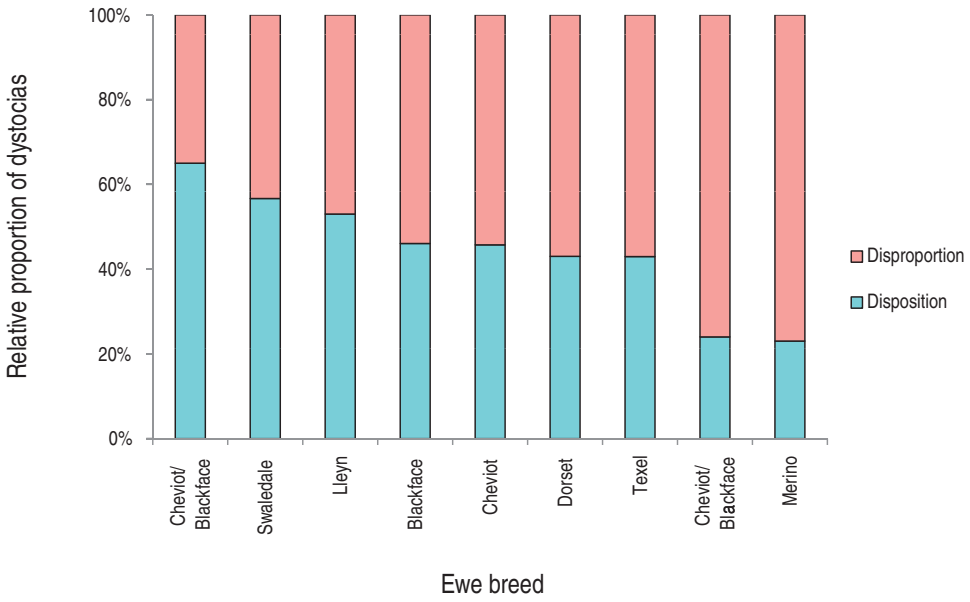

• **Fig. 12.3** Relative contribution of fetomaternal disproportion and faulty fetal disposition as causes of dystocia in ewes. (Data from Gunn 1968, Whitelaw & Watchorn 1975, George 1975, 1976, Speijers et al. 2010.)

primiparous ewes are more likely to experience dystocia due to disproportion than are pluriparous ewes. Grommers et al. (1985) noted that 5.7% of lambs were in posterior presentation – a figure that was remarkably similar to the 3.6% recorded by Wallace (1949) many years earlier. Difficult parturition occurred in nearly 65% of births in which the lamb was in posterior presentation (Grommers et al. 1985). In the study of Dwyer & Bunger (2012), nearly 20% of all malpresentations were of a posterior nature, but, of these, there were more lambs in breech position (11.6%) than in uncomplicated posterior presentation (9.6%). Overall, flexions of the forelimb (carpal, shoulder) are the most common type of malpresentation (46%–55% of all malpresentations; Grommers et al. (1985), Dwyer & Bunger (2012)), although noting that ewes with unilateral shoulder flexion can often lamb spontaneously. Grommers et al. (1985) noted that only 37% of such presentations were associated with dystocia. The prevalence of deviation of the head varies from 2% (Wallace 1949) to 6% (Dwyer & Bunger 2012). Dystocia due to congenital defects of the fetus ('fetal monsters') occurred in 2% of births in the study of Woolliams et al. (1983), with arthrogryposis accounting for 1% (i.e., half) of these defects.

The most important maternal cause of dystocia in ewes is nondilation of the cervix (ringwomb): studies reviewed by Purohit (2006) show that 25% to 50% of cases of dystocia in ewes are due to cervical nondilation. Such figures may, however, overrepresent the true prevalence of the condition inasmuch as such dystocias cannot be relieved without veterinary intervention. Consequently, studies from veterinary schools' clinics show incomplete cervical dilatation as a major cause of dystocia, whose prevalence ranges from 35% to 70% of cases of dystocia presented for veterinary assistance (Thomas 1990, Sobiraj 1994, Kloss et al. 2002). It is difficult to compare results from different studies, because there were differences in the criteria used to diagnose this disorder. However, there is an impression that incomplete dilatation of the cervix in sheep is more prevalent now than it was 30 or 40 years ago. Other maternal causes are relatively uncommon.

Does. The prevalence of dystocia in does is difficult to ascertain, as most published series are of relatively small numbers of animals. However, the prevalence is generally held to be relatively low: for example, Palanivel and Gajendran (2006) estimated it at 2.5%. Sobiraj (1994) noted that the main indication for manual assistance with kidding was abnormal fetal presentation, whereas the main indication for Caesarean surgery was fetomaternal disproportion. Cervical nondilation is a significant cause of dystocia in does: a study by Majeed and Taha (1989) found that cervical nondilation accounted for 25% of cases of dystocia, whereas Anusha et al. (2016) reported that it was the cause of 80% of maternal dystocia. Uterine torsion is generally considered to be relatively rare in the doe (Purohit 2006), although Philip et al. (1985) recorded it as the most common indication for Caesarean surgery in their series of cases. Faulty disposition of the fetus is the most common cause of fetal dystocia, accounting for between 60% (Purohit 2006) and 75% (Anusha et al. 2016) of such cases. In the study of Bhattacharyya et al. (2015), fetal dystocia (54%) was more common than maternal dystocia (37%). Head deviation, flexion of the forelimb, breech presentation, dog-sitting position, and fetal monstrosities were the common fetal causes, whereas cervical nondilation was the most common maternal cause.

Pig

Dystocia is generally considered to be less common in the pig than in monotocous animal species. Figures of 0.25%, 1% (Jones 1966, Jackson 1995), and 3% (Randall, 1972) have been reported in the literature. However, dystocia per se is of far less importance than is piglet survival through the perinatal period, so there is a limited focus upon dystocia as an entity.

In common with other polytocous species, maternal forms of dystocia are almost twice as common as fetal forms. Jackson (1972) reported that 37% of dystocias were caused by uterine inertia, 13% by obstruction of the birth canal, and 9% by downward deviation of the uterine horns, whereas 14.5% were caused by

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breech presentation, 10% by simultaneous presentation, 3.5% by downward deviation of the head, and 4% by fetal oversize. The incidence of fetal dystocia increases when the litter is small, because the size of the individual fetus tends to be relatively large and is therefore more prone to causing obstruction. Irregularities of limb posture and even uncomplicated posterior presentation often cause dystocia when the litter is small, whereas had the litter been large and its individuals small, these irregularities would not have interfered with normal expulsion. Monstrosities are not uncommon: they are generally of the double type, but schistosomes, perosomus, and hydrocephalic specimens also occur. Together, bladder flexion and vaginal prolapse were reported to be the third most common cause of maternal dystocia in a study in Germany (Schulz & Bostedt 1995), whereas hypocalcaemia should also be considered as a cause of uterine inertia (Framstad et al. 1989).

Horse

There are relatively few studies that provide reliable information on the prevalence and causes of dystocia in the mare, although the prevalence is generally regarded as being low. For example, Vandeplassche (1993) quoted 4% for Thoroughbreds and trotters, 10% in Belgian draft horses (a figure which is relatively high due to fetal muscular hypertrophy), and 8% in Shetland ponies (because of their relatively large skull). Sabbagh et al. (2014) found that normal foalings occurred in 91% (Ardennais) to 95% (Breton and Comtois) of heavy horse foalings, with 1% to 2% of foalings requiring veterinary assistance. McCue and Ferris (2012) found that unassisted delivery occurred in 87% and dystocia in 10% of Thoroughbred and Quarter Horse foalings. Ginther and Williams (1996) reported that the prevalence of dystocia in Quarter Horses, Standardbreds, Thoroughbreds, and Miniature Horses was 16%, 10.5%, 8.9%, and 19%, respectively. Similarly, Rosales et al. (2017) found a prevalence of 8.4% in Thoroughbred foalings. The prevalence of dystocia is higher in primiparous than in pluriparous mares, although mares that are older at their first parturition tend to have a lower risk of dystocia than those which are younger (Sabbagh et al. 2014).

It is widely accepted that the majority of cases of equine dystocia are of fetal origin, with abnormalities of presentation, position, and posture typically outweighing fetomaternal disproportion. Thus, Ginther and Williams (1996) found that malposition of the body accounted for 23% and malposture of the extremities for 41% of equine dystocias. Frazer et al. (1997) similarly found that 87% of equine dystocias referred to a veterinary hospital were due to abnormalities of presentation, position, or posture. Curiously, however, in a recent survey of Thoroughbred foalings in New Zealand, only 28% of cases were due to malposture of head or forelimbs, whereas 44% were considered to be due to relative fetal oversize (Rosales et al. 2017). The latter figure is unusually high, with other surveys suggesting that fetal oversize is the cause of dystocia in only 0.8% (McCue & Ferris 2012), 2% (Frazer et al. 1997), 5% (Vandeplassche 1993, Byron et al. 2002), or 10% (Leidl et al. 1993) of cases.

The presentation of the fetus in normal and abnormal foalings is shown in Table 12.1, based upon the summarisation by Vandeplassche (1993) of 170,000 foalings. The fetus was in anterior presentation in 99% of normal births and in posterior presentation in most other normal births. However, posterior and transverse presentations were significantly overrepresented in cases of dystocia. Dorsosacral positions are more common than dorsoilial, but dorsopubic presentations are relatively uncommon. Transverse presentations are more commonly ventral than dorsal and more common in heavy horses than in light animals (Frazer et al. 1997: Table 12.2). Transverse presentation of the foal across the uterine body (either dorso-transverse or ventro-transverse) is well known; the transverse bicornual pregnancy, another form of transverse disposition in which the extremities of the fetus occupy the uterine horns, is notorious and peculiar to the equine species but, fortunately, very rare.

All forms of postural irregularity occur in the mare. The head and neck may be displaced laterally or downwards between the forelegs. Such displacements may be further complicated by rotation of the cervical joints. The limbs are frequently presented abnormally; one, several, or all of the joints of the limbs may be flexed, and the irregularities are classified according to their clinical significance as carpal flexion, shoulder flexion, hock flexion, and hip flexion. Such abnormalities of position and posture account for a large proportion of equine dystocias: Vandeplassche (1987) found that 58% of dystocias in which the fetus was in anterior

TABLE 12.1 Influence of fetal presentation on dystocia in 170,000 foalings in the mare

Presentation	Normal Foalings: n (%)	Dystocia Cases: n (%)
Anterior	168,130 (98.9%)	408 (68%)
Posterior	1,700 (1.0%)	95 (16%)
Transverse	170 (0.1%)	98 (16%)

From Vandeplassche (1993)

TABLE 12.2 Presentation and position of 150 referred dystocias

	PRESENTATION					
	Anterior		Posterior		Transverse	
Position	All	107 (76%)	All	20 (14%)	All	14 (10%)
	Dorsosacral	73 (68%)	Dorsosacral	11 (55%)	Ventral	12 (86%)
	Dorsoilial	21 (29%)	Dorsoilial	8 (40%)	Dorsal	2 (14%)
	Dorsopubic	13 (12%)	Dorsopubic	1 (5%)		

From Frazer et al. (1997)

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presentation were associated with flexion of the neck; McCue and Ferris (2012) found that one or both forelimbs were flexed in 30% of dystocias and that the neck was flexed (laterally or ventrally) in 4% of dystocias; Frazer et al. (1997) reported that 56% of malpostures were due to lateral or ventral flexion of the neck and that 51% had flexion of forelimb joints (noting that some fetuses had more than one abnormality, so the total is >100%); and Ginther and Williams (1996) recorded that forelimb flexion accounted for 67% of malpostures, retention of the head 8%, and abnormalities of both head and forelimb(s) a further 17% of dystocias. Dystocia in which the fetus is in posterior presentation are as likely to be in breech or bilateral hock flexion posture as they are to be in an extended posture. Unilateral forelimb flexions appear less common than bilateral flexions, whereas unilateral hindlimb flexions are uncommon.

Dystocia due to fetomaternal disproportion is much less common than in cattle and is not normally the consequence of fetal oversize due to prolonged gestation. More cases of fetomaternal disproportion are due to a relatively small pelvis than an 'oversized' fetus, especially in younger, primiparous mares. Prolonged gestation in the mare is characteristically due to either placental or uterine insufficiency, resulting in a dysmature, rather than an overgrown, fetus (Frazer, 2011).

Maternal dystocia is generally less common than fetal: Leidl et al. (1993) found that 17% of dystocias were due to uterine torsion, 4% were due to incomplete dilatation of the birth canal, and 3% to ventral deflection of the uterus (these figures compare with 61% due to faulty fetal disposition). Vandeplassche et al. (1972) stated that uterine torsion accounts for 5% to 10% of severe cases of maternal dystocia, a figure that corresponds well with the 3% of cases among referred dystocias reported by both Frazer et al. (1997) and Karadjole et al. (2008). Gross fetal monstrosities are rare, but occasional developmental anomalies that cause dystocia are wryneck (fixed lateral deviation), hydrocephalus, and malformation of the fetal limbs. Wryneck is likely to occur with transverse bicornual pregnancy. Uterine rupture was present in 8% of the cases reviewed by Karadjole et al. (2008).

Dogs and Cats

Bitch

The literature provides relatively scanty details concerning the prevalence or causes of dystocia in the bitch. There are many reasons for this difficulty, not least because of the tendency for breeders to intervene (in some cases prematurely and unnecessarily) and their propensity to attempt the treatment of all but the most severe cases themselves. Also, there are great variations between breeds in the risks for dystocia, so data for prevalence and causes will therefore be greatly influenced by the population of animals in the study. Overall prevalence of canine dystocia has been estimated at 5% of all births (Linde-Forsberg 2005) and 2% of births in bitches in Sweden that had animal insurance (Bergstrom et al. 2006).

In the survey of Darvelid and Linde-Forsberg (1994), maternal causes were responsible for 75% of cases of dystocia and fetal causes for the remaining 25% (Table 12.3). Uterine inertia was by far the most important cause, accounting for 72% of cases. The authors separated uterine inertia into 'primary complete' inertia, in which the bitch failed to expel any pups (49%), and 'primary partial' inertia (23%), in which the bitch delivered at least one pup and then stopped before whelping was complete. Thus 'primary partial' inertia probably equates to 'secondary uterine inertia' of classic obstetrical parlance. Of the 15% of cases of faulty disposition of the fetus, 5% were due to transverse presentation, 4% were breech presentations, and 1% had neck flexion. In a survey of the causes of dystocia in India (Shwetha et al. 2014), the relative proportion of cases due to maternal (61%) and fetal (39%) causes were relatively similar, but the relative contributions to the causes of fetal dystocia differed: 24% of cases were due to abnormalities of head posture and 7% to abnormalities of hindlimb posture. Fetomaternal disproportion accounted for 5% of cases. Munnich and Kuchenmeister (2009) found a similar ratio between fetal (22%) and maternal (38%) causes, but also noted that both fetal and maternal factors existed in 35% of cases. Interestingly, in the latter study, human interference with labour contributed to dystocia in 43% of cases, and 2.5% of cases were due to iatrogenic uterine spasm due to oxytocin 'abuse'. When the effect of human interference is taken into consideration, uterine 'inertia' was again the predominant cause. Fetomaternal disproportion occurs as a result of a narrow birth canal or when fetal oversize is present (usually as a consequence of low/singleton litter size).

TABLE 12.3 Causes of dystocia in 182 bitches

Cause	No. of Cases (%)
Maternal	
Primary complete uterine inertia	89 (49%)
Primary partial uterine inertia	42 (23%)
Other maternal causes	6 (3%)
Total	137 (75%)
Fetal	
Faulty disposition	28 (15%)
Fetomaternal disproportion	12 (7%)
Fetal monster	3 (2%)
Fetal death	2 (1%)
Total	45 (25%)

From Darvelid & Linde-Forsberg (1994)

The effect of breed upon canine dystocia has been reported in several studies. Thus, for example, the Dachshund and Scottish Terrier are particularly prone to primary uterine inertia. The Corgi shows extreme variation in the size of its puppies and, hence fetomaternal disproportion may occur. The severe congenital deformities of achondroplasia and brachycephalism, which are the characteristics of some breeds, adversely affect the birth process. Achondroplasia results in a reduction in the dimension from the sacrum to the pubic bone and thus reduces the size of the pelvic canal. Brachycephalic breeds, particularly the Pug, together with the Sealyham and Scottish Terrier, are prone to obstructive dystocia due to the fetuses having comparatively large heads and the dams having narrow pelves. More recently, the Boxer has been identified as having an unusually high level of dystocia: Linde-Forsberg and Persson (2007) reported that dystocia occurred in 28% of Boxer whelpings. Dystocia was due to uterine inertia in 60% of cases, fetal malpresentation in 26%, and fetomaternal disproportion in 3%.

Large fetuses, causing fetomaternal disproportion, are commonly encountered in bitches gravid with only one or two pups.

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A primigravid bitch of the small breeds often has trouble expelling her first pup but, provided timely assistance is forthcoming, she usually expels the remainder of her litter normally. If, however, assistance is delayed, the onset of secondary inertia may make the outcome serious. Munnich and Kuchenmeister (2009) reported an interesting interaction between breed size and litter size, such that miniature and small breeds showed a high incidence of dystocia, but there was also a significant difference between the risks of dystocia in miniature breeds with litters of one to three puppies versus large breeds with more than eight puppies.

Abnormalities of position are common in both anterior and posterior presentation. Failure of the fetus to rotate before presentation results in its engaging in the pelvic inlet in a ventral or lateral position, thereby causing dystocia. Traverse presentation is rare and, when it occurs, the bitch generally has a bicornual gestation with a single fetus: this presentation is generally accompanied by uterine inertia. Irregularities of limb posture are generally of little importance provided the puppy is of normal size. In fact, many puppies are born with their fore- or hindlimbs flexed. However, when the fetus is relatively large, these postural deviations may be the factor that causes dystocia. Not infrequently, a dam that attempts to expel a fetus with its forelimbs retained partially succeeds in that the head is born, but the thorax with the limbs becomes obstructed in the maternal pelvic inlet. Similarly, a fetus may have its hind parts born while its distended thorax is obstructing. Irregularities of head posture are common, and vertex presentation (in which the occiput is the part that first enters the pelvis) and lateral deviation of the head are frequently encountered. An interesting feature of the latter abnormality is that it often involves the last puppy to be born. Fetal hydrocephalus and anasarca occasionally occur, but other forms of monster are rare. Gross umbilical hernia (schistosomus) is seen in the achondroplastic canine types, as well as in kittens, but it is seldom a cause of dystocia.

Queen

Estimates of the prevalence of dystocia in the cat are largely based upon data from pedigree cats or breeding catteries; very much less information exists on the prevalence in nonpurebred domestic shorthaired cats than in the purebred and pedigree populations. Within these limitations the overall prevalence of dystocia in the cat has been estimated to be 6% (2928 litters from 735 queens: Gunn-Moore & Thrusfield 1995) or 7% (256 cat breeders: Holst & Frossling 2009) of births resulting in delivery by Caesarean surgery. Sparkes et al. (2006) reported that veterinary assistance had been sought in 15% of births (1056 litters), of which 8% also required Caesarean surgery. Holst et al. (2017) used insurance records of purebred cats to identify a prevalence of 67 cases of dystocia per 10,000 'cat years at risk' (CYAR). Of the cats identified with dystocia in that study, 56% underwent Caesarean surgery; there was a case fatality rate of 2%. The same study noted that the risk of dystocia in nonpurebred cats was only 22 cases per 10,000 CYAR, but this low prevalence was attributed to the fact that domestic cats are 'generally not used for intentional breeding, leading to a comparatively low population at risk' (p44).

Maternal causes of dystocia are more common than fetal causes. In the study of Ekstrand and Linde-Forsberg (1994), maternal causes, chiefly uterine inertia, accounted for 67% of dystocias (Table 12.4). Fetomaternal disproportion and faulty disposition were the most common fetal causes. Posterior presentation, which occurs in 40% of kitten births, does not cause dystocia per se, but breech presentation does (Ekstrand & Linde-Forsberg 1994).

TABLE 12.4 Causes of dystocia in queens

Cause	No. of Cases (%)
Maternal	
Uterine inertia	94 (61%)
Fetomaternal disproportion due to narrow birth canal	8 (5%)
Other maternal causes	2 (1%)
Subtotal	104 (67%)
Fetal	
Faulty disposition	24 (16%)
Fetal congenital defects	12 (8%)
Fetomaternal disproportion due to fetal oversize	3 (2%)
Fetal death	7 (5%)
Subtotal	46 (30%)
Other causes	5 (3%)
Total	155

From Ekstrand & Linde-Forsberg (1994)

Head flexion is the most common faulty disposition associated with anterior presentation.

Various studies have examined the effect of breed upon dystocia. Sparkes et al. (2006) found no effect of breed upon the risk of delivery by Caesarean surgery. Conversely, Ekstrand and Linde-Forsberg (1994) found that, in a large colony of cats of mixed breeding, the frequency was 0.4%, whereas in litters of Devon Rex it was 18.2%. Pedigree litters were at a significantly higher risk than cats of mixed breeding (the odds ratio being 22.6). In the purebred populations studied by Holst et al. (2017), British Shorthair and Oriental queens had a higher risk of dystocia than other breeds (Fig. 12.4). Gunn-Moore and Thrusfield (1995) identified that dolichocephalic and brachycephalic types have a significantly higher level of dystocia than mesocephalic types. This has been attributed to the large, wide, flat-faced head of the Persian kitten (Wilkinson 1984).

Aetiology

Fetomaternal Disproportion in Cattle

The causes of bovine dystocia have been widely explored in the literature (McDermott et al. 1992; Table 12.5). Calf birth weight (an 'intermediate' cause according to the schema of Mee (2008)) is the key fetal factor in determining the probability of dystocia, whereas the key maternal intermediate causes are parity and size. Importantly, these factors appear to pertain widely across many immediate causes of dystocia; thus although their contribution to fetomaternal disproportion may be readily apparent, they also are the most strongly associated intermediate causes of dystocia due to fetal maldisposition.

Fetomaternal disproportion occurs due to a mismatch between the birth weight and conformation of the calf and the size and conformation of the dam. Factors which determine calf birth weight

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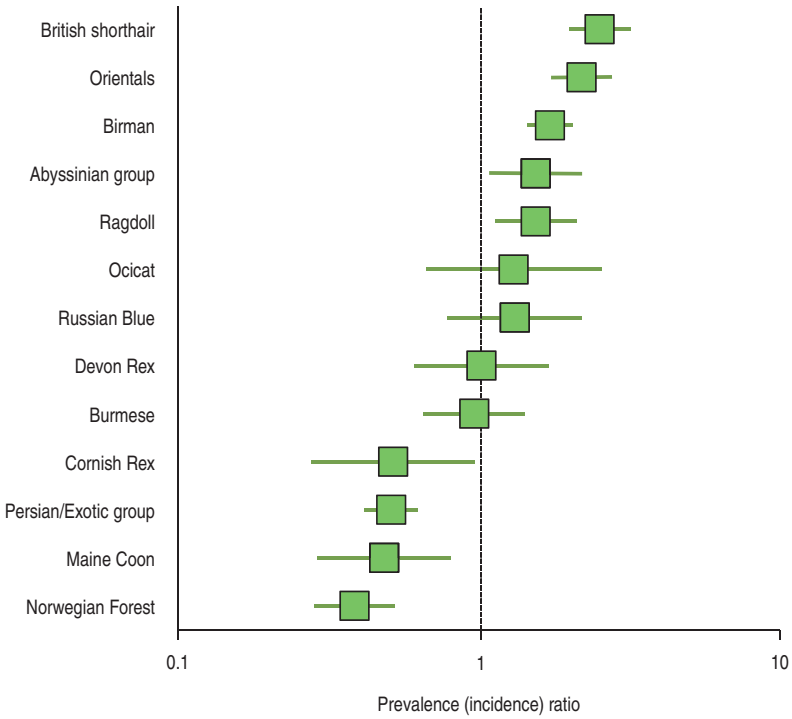

• **Fig. 12.4** Relative risks of dystocia in different breeds of cats compared with all other purebreds. Error bars represent the 95% confidence interval. (From Holst et al. 2017.)

TABLE 12.5 Influence of parity, calf sex, twins, and breed of sire and dam on the frequency of dystocia in cow–calf herds in Ontario

Factor	Total Number of Calvings	Easy Calvings (%)	Difficult Calvings (%)
Twins	73	13.7	4.1
Singles	4296	5.2	3.4
Primiparous	667	14.1	12.3
Pluriparous	3702	3.8	1.8
Heifer calf	2083	4.4	2.2
Bull calf	2065	6.1	4.5
Breed of Dam			
Hereford	1186	4.0	2.4
Limousin	264	4.2	1.1
Charolais	284	6.0	3.2
Simmental	354	11.6	6.5
Breed of Sire			
Hereford	1056	4.3	2.9
Limousin	1236	4.9	2.4
Charolais	896	5.6	3.3
Simmental	729	8.8	6.2

From McDermott et al. (1992)

TABLE 12.6 Factors affecting the risk of dystocia in Holstein cattle

Factor	Effect Upon Odds Ratio for Dystocia
Season	15% higher in winter than summer
Calf sex	25% higher in males than females
Parity	4.7 times higher in first than later parities
Calf birth weight	13% increase per kg increase in birth weight
Dam's pelvic area	11% decrease per dm^2 increase in pelvic area

From Johanson & Berger (2003)

and conformation include the breed and conformation of the sire, gestation length, sex of the calf, and single *versus* multiple pregnancy. *In vitro* production of calf embryos can result in a 'large calf syndrome' in a proportion of such fetuses. The primary maternal factor that determines the risk of fetomaternal disproportion is the parity of the dam. This is further related to the age, weight, and skeletal maturity at first calving. Pelvic conformation, particularly narrowness of the pelvic inlet and the length of the sacrum, as well as the presence of intrapelvic fat, affect both primiparous and multiparous cows. Plane of nutrition affects both dam and calf through all of the aforegoing, as well as directly.

Many studies show an increase in the risk of dystocia as birth weight increases, although calf birth weight is itself determined by the sex of the calf, the breed of its sire, its gestation length, and environmental factors, such as season and year (Table 12.6). However, the relationship is not entirely straightforward, for the relationship between calf birth weight and difficult calving differs markedly between primiparous and multiparous animals (Fig. 12.5),

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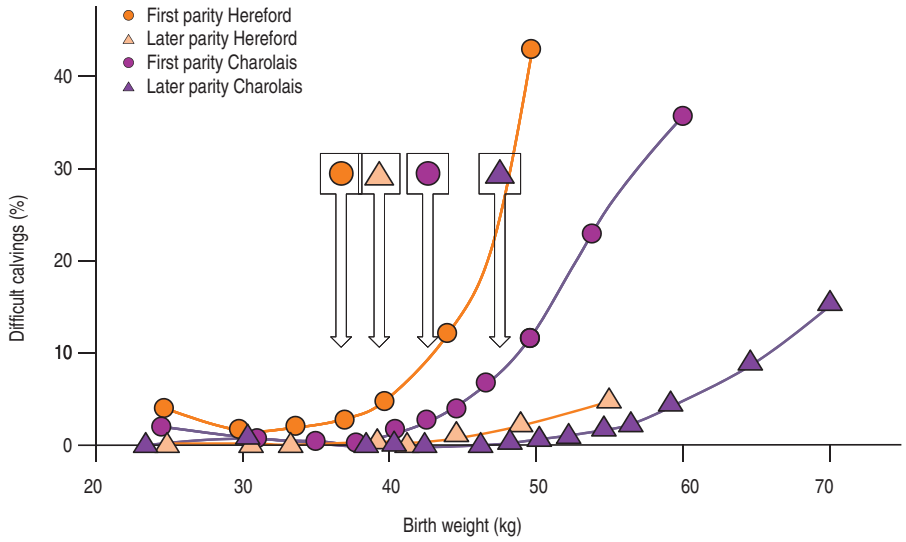

• **Fig. 12.5** Phenotypic relationships between calving difficulty and birth weight illustrated by weighted averages within birth weight intervals. Mean birth weights are marked as: ● first parity Hereford, △ later parity Hereford, ● first parity Charolais and later parity Charolais ▲ Mean birth weights are marked with vertical arrows. (Redrawn from Eriksson et al. 2004.)

TABLE 12.7 Effect of maternal liveweight and calf weight on calving difficulty in Friesian heifers

Heifer Live Weight After Calving (kg)	Calf Weight (kg)	Calving Difficulty (%)
384	36.5	48
482	38.5	26
549	41.1	24

From Anonymous (1997). The *Australian Cattle Veterinarian* 4:17.

TABLE 12.8 Relative frequency of severity of dystocia and birth weight and sex of calf

Birth Weight (kg)	Sex of Calf	Total Number of Calvings	Assisted (%)	Difficult (%)
20	M	23,949	11.3	2.2
20	F	25,069	6.4	1.1
21–25	M	3,085	5.9	1.0
21–25	F	5,588	3.9	0.4
26–30	M	13,023	9.3	1.3
26–30	F	19,118	6.6	0.8
31–35	M	21,165	16.4	3.5
31–35	F	19,368	11.5	2.2
36–40	M	10,372	29.0	10.1
36–40	F	5,007	23.7	7.0
40	M	2,164	33.6	27.8
40	F	542	30.0	20.6

From Berger et al. (1992)

such that the threshold at which the effect of increasing birth weight causes a significant increase in the risk of dystocia is much lower in primiparous than multiparous animals (Eriksson et al. 2004). The relationship is also compounded by the effect of litter size, such that, even though the birth weight of twins and triplets is lower than that of singletons, the strong relationship between multiple births and dystocia is such as to negate the effect of the lower birth weight of the multiple calves. To complicate the issue further, birth weight of calves born to primiparous animals increases as the weight of the heifer increases, but the incidence of dystocia decreases (Moran 1997), largely due to an improvement in the ratio between the dimensions of the dam and those of the calf (Johanson & Berger 2003; Tables 12.6 and 12.7).

Calf Birth Weight

Calf birth weight is the single most important fetal factor affecting the risk of dystocia in heifers (Meijering 1984, Morrison et al. 1985, Johnson et al. 1988), such that the larger the calf, the greater the chance of a difficult calving (Table 12.8). Linear estimates have shown that each kilogram increase of birth weight increases the rate of dystocia by 1.6% (Laster et al. 1973) to 13% (Johanson & Berger 2003). However, other studies have shown a nonlinear relationship between birth weight and dystocia, with the incidence of dystocia increasing significantly after the birth weight reached a certain threshold (Meijering 1984, Rice 1994). The threshold is determined chiefly by the parity and breed of the dam, as shown in the study of Eriksson et al. (2004) (see Fig. 12.5). Figures for the threshold therefore vary from study to study (which also varies with the definition of dystocia that was used in the study), so figures vary widely from approximately 30 to approximately 50 kg birth weight.

The relationship between birth weight and risk of dystocia is further complicated by the fact that the fetus does not grow homogenously but that the whole period of fetal development is characterised by sequences of positive and negative allometric growth of different parts of the fetal body. For example, the distal

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TABLE 12.9 Influence of parent on birth weight

Parent	Calf Weight (kg)
Purebred South Devon	45.4
Purebred Dexter	23.7
Calculated mean	*34.5*
Dexter bull x South Devon cow	33.2
South Devon bull x Dexter cow	26.7

Based on Joubert & Hammond (1958)

parts of the fetal limbs achieve peak growth rate earlier in gestation than do the proximal parts. Likewise, fetal muscle growth and intermuscular fat deposition is maximal in late gestation (Johnson 1974). Moreover, the growth of the fetus follows an exponential growth curve, with the greatest absolute increase in body weight occurring in the last trimester (although the highest relative growth occurs in the first trimester (Hickson et al. 2006)). Growth occurs by hyperplasia (mainly in early pregnancy) and hypertrophy (increasingly as pregnancy advances). Thus fetal growth can be retarded and, possibly, birth weight reduced by underfeeding of the dam in early gestation, but fetal growth rate is largely unaffected by the rate of maternal weight gain (Hickson et al. 2006).

Calf birth weight is, according to the schema of Mee (2008) an 'intermediate' cause of dystocia that itself depends on its own causal factors. Some of the 'ultimate' causes of birth weight are discussed here.

Breed of Sire. The effect of breed of sire upon the birth weight of the calf has long been recognised. Classic studies showed that the breed of both sire and dam contribute to the birth weight. Thus, when a Friesian bull is crossed with a Jersey cow, the birth weight approximates to the mean birth weight of the two breeds; but when a Jersey bull is crossed with a Friesian cow, the birth weight of the calf more closely approximates to that of the dam (Hilder & Fohrman 1949). Similar effects occur in the more extreme cross between the South Devon and Dexter breeds (Joubert & Hammond 1958: Table 12.9).

There have been many subsequent surveys that have shown similar results. In beef cows Laster et al. (1973) showed that Angus and Hereford cows bred by Simmental, South Devon, Charolais, and Limousin bulls experienced significantly more dystocia (32%, 32%, 31%, and 31%, respectively) than those bred by Hereford, Angus, or Jersey bulls (16%, 10%, and 6%, respectively). In 1986 Cundiff et al. showed similar breeds as being most commonly associated with high birth weight and hence dystocia. These data, along with the associated effects of sire breed upon gestation length are shown in Table 12.10. The strength of the relationship between birth weight and dystocia had, by that time, become so well established, that selection programmes were introduced into many beef breeds to try to reduce birth weight while not adversely affecting postnatal growth rate. The results of these programmes were illustrated by Ahlberg et al. (2016), who showed that the larger beef breeds (calves sired by Simmental, Charolais, Limousin, Maine-Anjou bulls) had fewer calving difficulties than would have been expected from the situation 30 years previously.

The effect of dairy sire upon the birth weight of dairy calves depends on the breed and parity of the dam, such that calves sired

TABLE 12.10 effect of sire upon birth weight, gestation length, and calving difficulty of calves born to Hereford, Angus, or Hereford–Angus dams

Breed of Sire	Birth Weight (kg)	Gestation Length (days)	Difficult Calvings (%)
European Beef Breeds			
Hereford–Angus	35.7	284.0	2.9
Limousin	38.9	289.2	9.4
South Devon	37.7	286.7	11.9
Simmental	40.3	287.3	14.9
Charolais	41.1	287.0	18.4
Maine Anjou	41.1	285.3	20.4
***Bos Indicus* and Crosses**			
Brangus	36.7	285.5	3.9
Santa Gertrudis	38.9	286.0	4.5
Sahiwal	38.0	294.0	6.2
Brahman	40.9	291.7	10.0
Dairy Breeds			
Jersey	31.1	282.9	2.9
Holstein	37.0	282.0	5.1
Brown Swiss	38.8	285.0	8.4

Based on Cundiff et al. (1986)

by Jersey bulls have low birth weights and a low proportion of assisted calvings, which is remarkably unaffected by parity, whereas calves sired by Holstein bulls have higher birth weights and a much higher proportion of assisted calvings, especially in first parity dams (Table 12.11). This well-recognised phenomenon is widely exploited among dairy farmers when choosing a sire (i.e., Jersey) to use for breeding maiden heifers to minimise the risk of dystocia at their first calving. Interestingly, the results of similar reciprocal cross experiments between Holsteins and Brown Swiss or Norwegian Red have also shown that these other breeds, and their crosses with Holsteins, had lower dystocia and/or stillbirth rates than did purebred Holsteins (Heins et al. 2006, Mee et al. 2011, Yao et al. 2014).

Finally, the effect of beef breed sires upon birth weight and dystocia of calves born to dairy cows has been extensively studied in the UK dairy herd (McGuirk et al. 1998b; Table 12.12). Again, the large breeds (Charolais, Limousin, Blonde d'Aquitaine, Simmental and Piedmontese) are associated with higher levels of dystocia, particularly in heifers, than are the smaller Angus and Hereford. The Belgian Blue presents an interesting case, however. The 'double muscling' of Belgian Blue calves is commonly associated with dystocia, particularly in purebred animals (Fiems et al. 2001). Conversely, McGuirk et al. (1998a) found that the prevalence of dystocia in dairy heifers and cows bred to Belgian Blues was only 1.6% and 3.1%, respectively. A recent study in Poland (Przysucha et al. 2013) also found that the prevalence of assisted calvings (10.3%) and very difficult calvings (3.2%) of Belgian Blue–Holstein calves was no different to that of difficult calvings in purebred Holstein cows.

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TABLE 12.11 Effect of sire and dam breed upon birth weight and calving difficulty of calves born to first or second parity Jersey or Holstein cows

Sire	Holstein		Holstein		Jersey		Jersey	
Dam	Holstein		Jersey		Holstein		Jersey	
Parity	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Assisted calvings (%)	36.0	11.6	19.1	12.8	2.1	5.5	0.0	1.9
Birth weight (Kg)	39.5	42.7	30.9	34.8	32.2	35.1	23.4	27

Derived from Olson et al. (2009)

TABLE 12.12 Incidence of difficult calvings according to breed of sire and parity of dairy cows and heifers

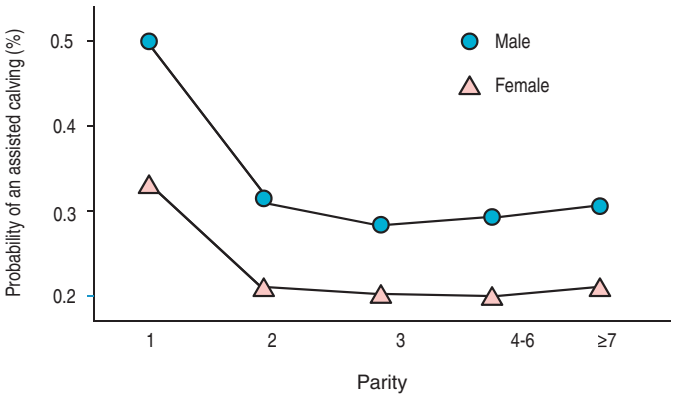
	Seriously Difficult Calvings (%)	
Sire Breed	Heifers	Cows
Aberdeen Angus	3.5	2.8
Hereford	5.0	1.3
Charolais	5.8	3.8
Limousin	6.3	2.1
Blonde d'Aquitaine	8.1	3.8
Simmental	8.3	3.8
Piedmontese	10.2	2.8
Mean	**6.0**	**2.9**

Data from the Belgian Blue breed are omitted from this table and are discussed in the text.
Derived from McGuirk et al. (1998b)

TABLE 12.13 The relationship between gestation length and calving ease in Holstein–Friesian cows

	Gestation length (days)				
Calving Ease	<275	275–277	278–280	281–283	>283
Unassisted	56.0	73.1	66.7	62.1	51.1
Little intervention	29.5	15.8	22.9	23.1	25.8
Difficult	9.7	6.8	10.5	8.7	16.6
Very difficult	4.9	4.3	5.4	6.1	6.6

Derived from Nogalski & Piwczyński (2012)



• **Fig. 12.6** Interaction between calf sex: male ●, female △, and parity on the odds of an assisted calving. (Based upon Mee et al. 2011.)

Sex of Calf. Many studies have shown, irrespective of breed, that birth weights of male calves are greater than female calves. The study of Johanson et al. (2011), which showed a mean birth weight of 38.6 (SD 5.3) kg for female calves versus a mean of 41.7 (SD 5.7) for males, would be typical of such studies. This increase in weight results in an increase in the risk of dystocia in male calves: Johanson and Berger (2003) estimated that the odd of a dystocia is 25% greater for a male than a female calf (Table 12.6). The relationship between calf sex, parity and dystocia is shown in Fig. 12.6.

Length of Gestation. Gestation length does vary between breeds and between individual sires (dams) within breed (see Table 12.10 for examples). It also varies with the sex of the calf, such that male calves usually have a few days longer gestation than do female calves. Gestation length affects the risk of both dystocia and perinatal mortality/stillbirth directly and through its effects upon calf birth weight, but the two are not synonymous. Examples of the relationship between gestation length and calving difficulty for Holstein–Friesian calves are shown in Table 12.13.

The heritability of gestation length is relatively high: h^2 for direct selection has been variously estimated at between 0.2 (Nogalski & Piwczyński 2012), 0.5 (Johanson et al. 2011), and 0.6 (Mujibi & Crews 2014), with the h^2 for maternal selection being roughly an order of magnitude lower. The genetic correlations between gestation length, risk of stillbirth, calving ease, and calf birth weight are consistently reported as being high (McClintock et al. 2005, Jeyaruban et al. 2016), providing opportunity for genetic selection to improve on calving difficulty/stillbirth rate. Genetic effects upon maternal and fetal components of dystocia are discussed later in the chapter.

Season and Climate. Although many studies have investigated the influence of season of year and climatic factors on birth weights and the incidence of dystocia, there is a lot of disparity between results. For example, in the temperate climate of the UK, McGuirk et al. (1999) showed (Fig. 12.7) that calf size and calf conformation declined in autumn and early winter and that there was a relationship between the average calving difficulty score and gestation length. Similarly, Colburn et al. (1997) found that the mean spring birth weights of calves born after a warmer-than-normal winter were 4.5 kg lower than those after a cold winter; the corresponding levels of calving difficulty were 35% and 58%, respectively. The

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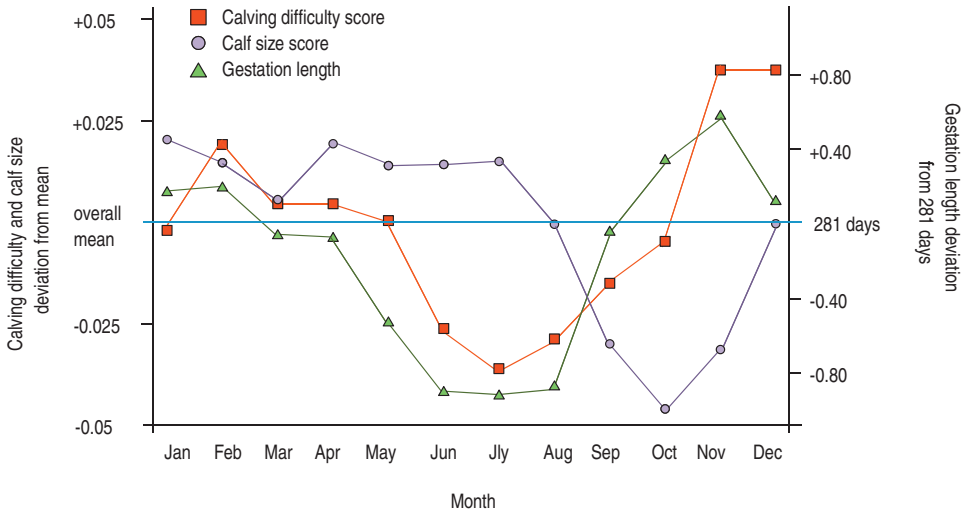

• **Fig. 12.7** Effect of month of calving on calving difficulty score ■, calf size score ●, and gestation length ▲ of cows in UK dairy herds. (Redrawn from McGuirk et al. 1999.)

TABLE 12.14 Effect of parity on calf birth weight and ease of calving in Holstein cattle

	Parity				
	1	2	3	4	≥5
Body weight of cow (kg)	489.4	553.1	595.2	610.7	629.7
Birth weight of calf (kg)	37.9	39.7	41.1	41.1	41.6
% unassisted calvings	48.3	79.9	82.7	82.8	86

Based on Sieber et al. (1989)

reduction in gestation length and increased calving difficulty were slightly out of phase with and preceded increase in calf size. In a more extreme winter climate Ugurlu et al. (2014) found that calf birth weight was lowest during the coldest part of the winter. In the semiarid climate of Sudan, Ali et al. (2015) showed that beef-breed calves that were born in the winter season had the highest birth weight, followed by those born in dry summers, whereas calves born in wet summers had the lowest birth weight. The findings of El-Nazeir et al. (2015) in Holstein crosses were not dissimilar, in that the birth weight of calves born in wet summers and the winter were higher than those born in dry summers. Mee (2008) summarised the potential mechanism for the effect of cooler weather upon birth weight as follows: 'cold weather … during the last trimester has been associated with increased dry matter intake, increased thyroid hormone concentration, increased blood and nutrient flow to the uterus and increased gestation length and reduced plasma oestradiol concentrations leading to increased birth weight and dystocia' (p97).

Effects of the Dam. Calf birth weight is affected by the age, parity, plane of nutrition, and body condition of the dam.

Birth weight of calves generally increased with both the age and the parity of the dam. Paradoxically, however, despite this increase in birth weight, the prevalence of dystocia decreases with both the age and the parity of the cow. Thus Sieber et al. (1989), from their study of Holsteins over an 18-year period (Table 12.14), showed that the body weight of the cows increased with parity, as did the birth weight of their calves, but the proportion of calvings that required assistance declined. Thus various authors have shown that heifers, which are older at their first calving, have a reduced risk of dystocia (Klassen et al. 1990, Berger et al. 1992, Hickson et al. 2009) than those which are younger, although it may be that as age increased beyond an optimal point the risk of dystocia increases. This, presumably, represents the interaction between the birth weight of the calf and the skeletal maturity of the dam.

The relationship between plane of nutrition of the dam and birth weight of the calf has also been of interest. Evidence from humans and laboratory animals suggests that permanent effects upon fetal (and, indeed, postnatal) growth rates can be induced by feed restriction in the first trimester of pregnancy. It has been postulated that it might be possible to exploit this phenomenon to reduce the birth weight of heifers' calves. However, neither Prior and Laster (1979) nor Hickson et al. (2009) were able to alter the birth weight of Angus calves by altering the plane of nutrition during the first trimester of pregnancy.

Effects of feeding later in pregnancy have also been extensively studied, but it is difficult to evaluate these results because much of it is contradictory (Meijering 1984, Hickson et al. 2006, and references therein). In summary, feed restriction of heifers in mid to late gestation commonly reduces calf birth weight, although without a consistent effect upon dystocia. This appears to be because feed restriction not only reduces the growth of the fetus, but also reduces the growth and skeletal maturity of the growing primiparous animals. Feed restriction of parous animals is much less consistent in its effect upon calf birth weight, with some studies showing a reduction in birth weight of calves born to feed-restricted dams and others showing no effect. The effect upon dystocia is either negligible or inconsistent. Heifers or older cows that are overfed during pregnancy can become obese; but although this seems to have no effect on birth weight (Arnett et al. 1971, Prior et al. 1979), calving performance and, consequently, stillbirth incidence may be affected negatively (Arnett et al. 1971, Lowman 1979) because of fat accumulation in the pelvic region (Grunert 1979, Lowman 1979).

The effects of the body condition score (BCS) of the dam on birth weight have been reviewed by Mee (2008) and Zaborski et al. (2009) (and see references therein). Mee noted that excess or inadequate BCS at calving in primiparous Holstein–Friesian cattle presents a significant risk factor for dystocia. He suggested an

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optimal BCS of 2.75 to 3.0 (0–5 scale) and attainment of 85% to 90% of mature bodyweight at first calving to minimise the risk of dystocia. Zaborski et al. (2009) also considered that BCS must be optimal to minimise the risk of dystocia and that both obese and thin cows have an increased risk of dystocia (and obese cows have a greater risk of metabolic disease in the peripartum period as well).

Taken together, it seems that attempting to manipulate calf birth weight by altering the plane of nutrition of the dam is a relatively unrewarding exercise. Excess feeding does not consistently increase calf birth weight, but can result in dystocia. Underfeeding may reduce calf birth weight in primiparous animals (although the effect is inconsistent in multiparous animals), but any benefit of reduced birth weight seems to be offset by deleterious effects upon the growth of the dam, resulting in an overall increase in the risk of dystocia.

Abnormal Offspring Syndrome (AOS). The use of *in vitro* matured (IVM), *in vitro* fertilised (IVF), and somatic cell nuclear transfer (SCNT)-derived embryos (see Chapter 44) has increased substantially in recent years, particularly in cattle, with great advances in this biotechnology. However, one of the disadvantages is that a proportion of the embryos derived from these methods have various developmental anomalies, collectively known as the 'abnormal offspring syndrome' (AOS) (Farin et al. 2006, 2015).

An increase in birth weight has been widely reported in calves derived from assisted reproductive technology (ART)-generated embryos. For example, Behboodi et al. (1995) reported 51 kg versus 36 kg, Kruip and den Daas (1997) a 4.5 kg higher birth weight, and van Wagtendonk-de Leeuw et al. (1998) a 10% increased birth weight. This increase in birth weight has been associated with a 2 to 3 day increase in gestation. The consequence is an increase in the dystocia rate and calf mortality rate; for example, Kruip and den Daas (1997) reported 25.2% dystocia and Behboodi et al. (1995) 62%, compared with 10% for artificial insemination (AI)-derived calves. The manifestation of AOS as increased fetal birth weight is, however, only one part of the syndrome: gestational failure in all stages of pregnancy and abortion; musculoskeletal deformities, disproportionate fetal growth; abnormal organ growth and physical/metabolic abnormalities that persist into postnatal life; abnormalities of placentation, including abnormal development of the allantois, placental vasculature, and, perhaps, hydrallantois – these are all part of the AOS. Current research into the mechanisms that result in this wide range of abnormalities has been reviewed by Farin et al. (2015).

Calf Conformation

The ability of a calf to be expelled unaided through the birth canal at parturition is dependent on its shape or conformation. This is seen in the most extreme situation of some fetal monsters (see Chapters 9, 14 and 15), such as fetal duplication, schistosomes, ascitic and anasarcous calves (in which the weight of the fetus is low but the conformation prevents normal expulsion), and in the condition of muscular hypertrophy (in which the muscle mass of the calf is increased disproportionately to its weight).

Muscular Hypertrophy ("Double Muscling"). Certain breeds of cattle, mainly beef, have a high frequency of muscular hypertrophy (double muscling). The condition is of genetic origin, resulting from mutations of the myostatin (Growth Differentiation Factor 8) gene (Grisolia et al. 2009). The highest frequency occurs in the Belgian Blue and Piedmontese breeds, but it also occurs in other breeds, including the Blonde d'Aquitaine and Charolais. Muscular hypertrophy is beneficial inasmuch as it results in a beef carcase that has a greater muscle content and, hence, meat of high lean content and high quality. However, the frequency of dystocia associated with double muscling is very high. Hanzen et al. (1994) and Fiems et al. (2001) both reported that 90% of pure breed Belgian Blues are delivered by Caesarean surgery, whereas Murray et al. (1999) reported a figure of 82% elective Caesarean surgeries in the UK. However, Arthur et al. (1989), who examined the effects of reciprocal crossbreeding of double muscled and normal sires and dams, found that the incidence of dystocia was highest when double muscled sires and dams were bred together, but that breeding a double muscled sire to a normal dam resulted in an incidence of dystocia that was not different from normal sire–dam breeding. This was interpreted in terms of the shape of the pelvis of the dam versus the muscular hypertrophy of the animal; thus the higher width of calves with hypertrophy causes dystocia when birth occurs through the more narrow pelvic opening of cows that themselves have muscular hypertrophy. These data are illustrated in Table 12.15. Zaborski et al. (2009) noted that, as muscular hypertrophy is caused by mutations of the myostatin gene, the genotyping and the appropriate mating management may reduce the dystocia rate.

TABLE 12.15 Reciprocal breeding between double muscled and normal cattle on the incidence of dystocia and perinatal mortality

Mating (Sire X Dam)	Calf Birth Weight (kg)	Difficult Calvings (%)	Perinatal Mortality (%)
DM x DM	31.9	26.7	13.4
N x N	36.7	9.9	4.6
DM x N	34.7	9.5	6.6
N x DM	34.8	18.2	7

DM, Double muscled; *N*, normal.
Derived from Arthur et al. (1989)

Conformation of the Calf. Attempts have been made to assess the conformation of normal calves and to correlate this with ease of calving. Such methods have involved asking the farmer to assess the conformation of the calf as good, average, or poor, and then applying a numerical score from 1 to 3 to each subjective value (McGuirk et al. 1998a). Others have made a large number of fetal anatomical measurements, such as head circumference, foot circumference, width of shoulders, width of hips, depth of chest, body length, cannon bone length, and diameter (Nugent et al. 1991, Colburn et al. 1997). Using the simple approach, McGuirk et al. (1998a) found a statistically significant difference between calf conformation and incidence of difficult calvings and calf mortality (Fig. 12.8). In summary, well-muscled calves born from a beef sire and dairy cow or heifer resulted in more difficult calvings and increased calf mortality. Recently, in Australia and New Zealand there has been emphasis on advising farmers on conformation when they purchase new beef sires (Hickson et al. 2006). They are advised to avoid bulls that are wide at the point of the shoulder or have a wide space between the tops of the scapulae.

The results from more sophisticated measurements of fetal conformation have been contradictory. Neither Meijering (1984), Morrison et al. (1985), Sieber et al. (1989) nor Barrier et al. (2013) found any relationship between calf body measurements on ease of calving, except inasmuch as they affected birth weight, although Barrier et al. did find an association between calf conformation and

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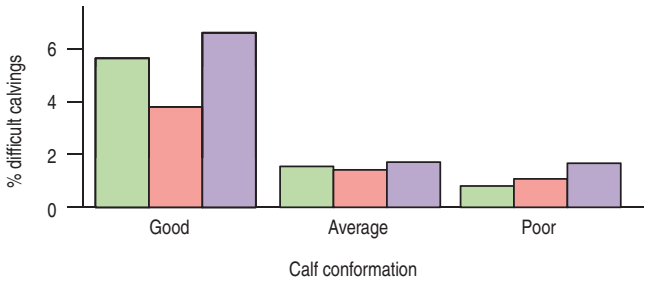

• **Fig. 12.8** Relationship between the incidence of difficult calvings and calf conformation for calves sired by Limousin, Hereford, and Charolais bulls. (Redrawn from McGuirk et al. 1998b.)

stillbirth that was independent of effects upon dystocia. Nugent et al. (1991), who examined the relationship between calf shape and sire, expected progeny difference (EPD), or ease of calving, found that at constant birth weight, calves from higher birth weight EPD bulls tended to have larger head and cannon bone circumferences. However, at constant birth weight, body measurements were not associated with calving ease. In conclusion, they stated that calf shape seemed to add no information for the prediction of dystocia other than that provided by birth weight EPD.

Recent studies have, however, identified quantitative trait loci (QTL) that are variously correlated with the probability of dystocia, risk or stillbirth or fetal birth weight. Seidenspinner et al. (2011) associated pleomorphism of the placental growth factor gene with maternal calving ease and stillbirth rate, whilst Pausch et al. (2011) associated regions of Chromosomes 14 and 21 with paternal calving ease. Hoglund et al. (2012) identified a cluster of loci on Chromosome 18 (with other loci on Chromosomes 13, 9, 18, 3, and 1) that were associated with indices of paternal calving ease. Loci associated with adult stature and conformation have also been associated with calving ease: Saatchi et al. (2014) and Sahana et al. (2015) identified a cluster of genes on Chromosome 6 that are associated with maternal conformation, calf birth weight, and calving difficulty. More recently, Mao et al. (2016) have associated QTLs on Chromosome 18 with calving difficulty. Interestingly, these QTLs are also associated with maternal conformation, or are expressed in the placenta, or are involved in regulating the onset of labour, which, taken together, can be readily interpreted in terms of mechanisms that might affect aspects of birth weight and gestation length.

Thus the phenotypic relationship between dystocia, stillbirth, and measurable attributes of calf conformation remains difficult to quantify. Simple scores of 'difficult' conformation seem to be well associated with the likelihood and degree of dystocia, but attempts to characterise these malconformations in terms of the dimensions of specific body parts of the fetus have not yet succeeded. On the other hand, the relatively high heritability of dystocia traits and the recent identification of specific loci, and even genes, that are associated with fetal and maternal dystocia traits, seem to afford opportunity to better understand the aspects of calf conformation and birth weight that are critical to the risk of dystocia.

Maternal Factors

Parity. The effects of parity upon ease of calving have been reported in a great many studies; heifers generally have more assisted calvings and a greater degree of calving difficulty than parous cows. The degree of difficulty continues to decline as the parity of the animal increases, but the difference in the rate of dystocia between the first and second parity is usually much bigger than between second and subsequent parities (Zaborski et al. 2009). Parity is the most important maternal factor which affects ease of calving and, indeed, virtually every survey of the causes of dystocia highlights parity as a highly significant effect. In the present chapter, Tables 12.5, 12.6, 12.11, 12.12, and 12.14 and Figs 12.1 and 12.5 all show this effect. The disproportionately high rate of calving difficulty in Holstein heifers has also been noted.

TABLE 12.16 Relative risk of dystocia in Angus heifers at different ages

Age of Dam (months)	Sex of Calf	Calvings Assessed as: Needing Some Assistance (%)	Calvings Assessed as: Difficult (%)
≤23	M	21.0	6.2
	F	13.0	2.6
24–25	M	7.2	4.0
	F	8.1	2.1
26–27	M	6.0	3.0
	F	6.5	1.3
28–29	M	8.7	2.6
	F	5.5	1.4
>29	M	8.0	2.0
	F	4.4	0.7

From Berger et al. (1992)

Pelvic Capacity of the Dam. The dam influences the incidence of fetomaternal disproportion primarily through her pelvic area. Pelvic area, in turn, is influenced by genetic effects, such as sire and inbreeding, and environmental effects, such as parity, weight at insemination, age, weight, and body condition at calving (Mee 2008). Primiparous animals have relatively smaller pelvic areas than parous animals because of their smaller stature and the relatively late maturation of the pelvis (Hiew & Constable 2015). Thus heifers that are smaller or younger at first parturition are at greater risk of fetomaternal disproportion than are those that are larger or older (Table 12.16). The risk of dystocia is also, therefore, related to the size and weight of the heifer at parturition (see Table 12.6), as well as the ratio between the birth weight of the calf and the size of the pelvis of the heifer (Table 12.17). Breed-related differences in the risk of dystocia can also be related to pelvic area, inasmuch as the ratio between pelvic area and heifer weight, and between pelvic area and calf birth weight, is significantly greater for Jersey heifers than it is for Holstein heifers (Nogalski & Mordas 2012).

Pelvic area can be estimated from internal measurements of pelvic height (perpendicular distance from the cranial end of the pubic symphysis to the ventral surface of the midsacrum) and pelvic width (largest distance between the shafts of the ilia) (Meijering 1984). From these measurements pelvic area can be calculated as the simple product of height × width, or by either treating it as an ellipse or by equations that regress actual area on these two measurable dimensions. Measurements are made transrectally using callipers (which can be difficult to obtain in some circumstances). For this reason the validity of the measurements and, hence, the whole concept has been criticised (Van Donkersgoed et al. 1990). Pelvic area can also be estimated by external measurements of the

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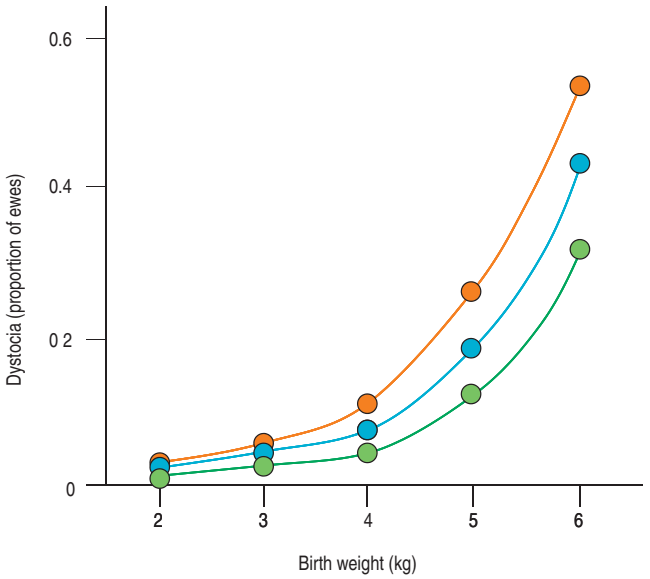
TABLE 12.17 Degree of dystocia in 2-year-old Hereford heifers

Degree of Calving Difficulty	Yearling Heifer Pelvic Area (cm^2)	Calf Birth Weight (kg)	Pelvic Area to Birth Weight Ratio[a]
1 (no assistance)	146	31.4	2.1
2 (slight assistance)	141	32.7	1.9
3 (moderate)	138	34.1	1.8
4 (much assistance)	142	37.7	1.7
5 (Caesarean operation)	132	36.8	1.6

[a]Yearling pelvic area divided by calf birth weight
Reference: Deutscher (1985)



• **Fig. 12.9** Relationship of dystocia at birth with birth weight for different sire breeds: Blackface, Swale, and Texel. (Redrawn from Speijers et al. 2010.)

pelvis, stature, and girth of the cow, with reasonable correlations between such measurements and pelvic area (Hiew & Constable 2015). The correlation between pelvic area and dystocia ranges from approximately 0.1 to 0.45 in beef heifers (Meijering 1984, Johnson et al. 1988), although others (King et al. 1993) have been unable to show any significant relationship. Consequently, despite the relationship between pelvic area and dystocia, the use of pelvic area as a predictor of dystocia in individual animals is of limited value (Van Donkersgoed et al. 1990). There appear to be many reasons for this. First, once a threshold pelvic area has been exceeded, further increases in pelvic area have a very limited effect upon the risk of dystocia (Mee 2008). Second, the correlation between pelvic area before parturition with that at parturition is low (Hiew & Constable 2015), particularly as the shape and area of the pelvis can change substantially during parturition; Meijering (1984) noted that increased mobility of the iliosacral joints and relaxation of the pelvic ligaments can increase pelvic height by up to 5% at the time of calving, with further increases occurring during abdominal straining.

Measurements of pelvic area have been used to cull very small yearlings as a means of reducing dystocia at first calving (Holm et al. 2014). If pelvic area measurements are made before service, animals with a small pelvic canal can be rejected for breeding or inseminated with semen from an easy-calving bull (low EBV for birth weight), whereas those with a larger pelvis can be bred to an average-calving bull. The heritability of pelvic area is relatively high, so it is potentially amenable to genetic selection. There has also been interest in the use of pelvimetry in bulls in an attempt to select sires that have a large pelvic area that might then be inherited by their female progeny. Results, however, have been equivocal (Crow & Indetie 1994, Kriese et al. 1994). However, Bennett and Gregory (2001) were of the view that direct selection for calving difficulty score or calf birth weight would be preferable because of their superiority in predicted genetic change.

Condition Score of the Dam. It is generally accepted that heifers or cows with a very high condition score are more likely to suffer from dystocia than those that are moderate to poor, the reason being that those in very good condition will have a substantial amount of retroperitoneal pelvic fat, which will reduce the overall size of the birth canal. Studies in both beef and dairy cattle have shown that body condition score over midranges has little influence on the dystocia rate. Thus, Spitzer et al. (1995) showed that the birth weight of calves born to beef heifers whose condition ranged from 4–6 (0 (emaciated) to 9 (obese) scale) were 28.9, 30.4 and 32.4 kg, respectively. Such birth weights are usually not associated with dystocia. Keady et al. (2001) likewise showed no effect of variation in midrange conditions scores of dairy cattle upon dystocia rate or calf birth weight.

Fetomaternal Disproportion in Sheep

Fetomaternal disproportion in sheep occurs as a result of a large lamb or a small pelvis or, sometimes, the simultaneous occurrence of both.

Lamb Birth Weight

Lamb birth weight is determined by breed (see Gootwine (2005) for extensive data on different sheep breeds), number of lambs in the litter, season, and parity. As birth weight increases, so does the risk of dystocia, with the magnitude of the relationship being modified by breed (Fig. 12.9).

Examples of the variability between breeds are illustrated in Fig. 12.10. Many lambs are, however, crossbred rather than purebred, so the effects of crossbreeding upon lamb birth weight have been extensively studied. In the classic study of Hunter (1957), in which large (Border Leicester) and small (Welsh Mountain) breeds were crossed, the birth weight of Border Leicester lambs born to Welsh Mountain ewes was 1.1 kg less than that of Border Leicester lambs born to Border Leicester ewes; also, the birth weight of Welsh Mountain lambs born to Border Leicester ewes was 0.6 kg more than that of Welsh Mountain lambs born to Welsh Mountain ewes. Similar results were obtained in reciprocal embryo transfer experiments by Dickinson et al. (1962) (Welsh Mountain and Lincoln breeds) and Sharma et al. (2012) (Suffolk and Cheviot breeds), both of whom found that the size of the fetus was constrained by the maternal recipient breed. Thus the maternal influence can limit the size of a genetically larger lamb, as well as increase the size of a genetically smaller lamb. This relationship was quantified

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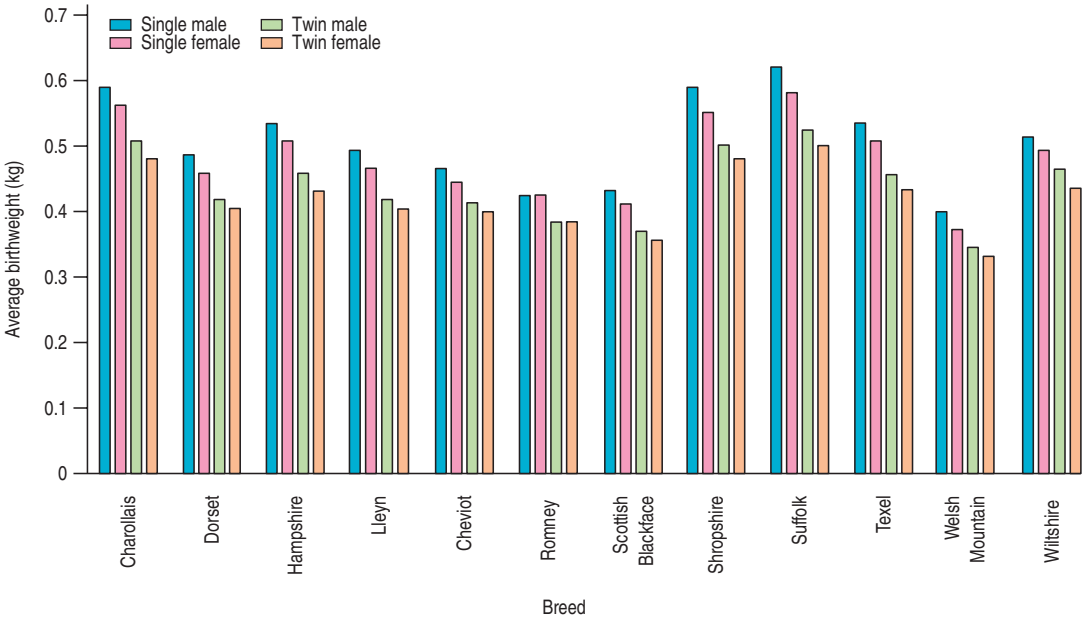


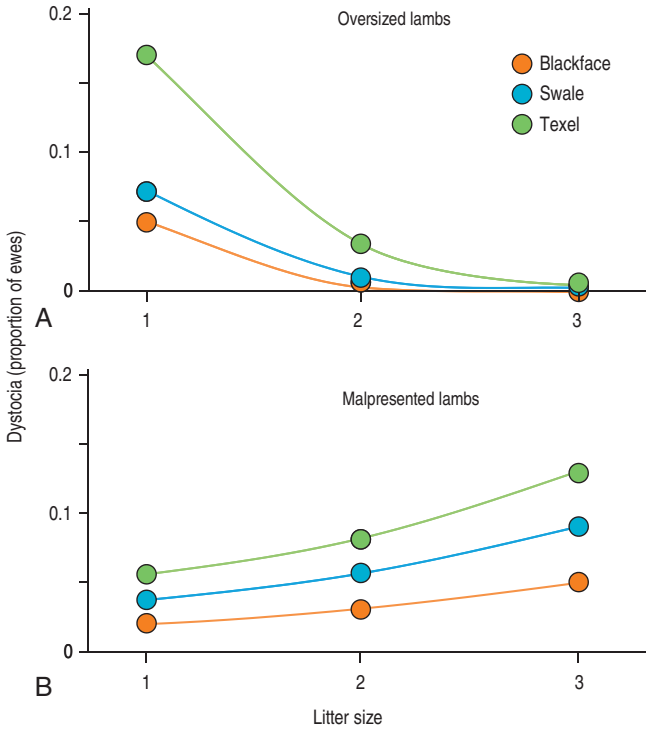
• **Fig. 12.10** Differences in average birth weight of lambs, according to sex, litter size, and breed. Single male ■, single female ■, twin male ■, twin female ■. (Modified from data obtained by Signet Breeding Services/AHDB Beef and Lamb and used with their permission.)

by Dickinson et al. (1962) as being related to the mature weight of the donor ($D^{0.83}$), the ratio of the weights of the donor and recipient ($(R/D)^{0.83}$), and the reciprocal of the litter size.

Litter size is closely related to birth weight, with single lambs being significantly heavier than multiples. Across a great many breeds, Gootwine (2005) showed that mean birth weights for single lambs was 4.6 ± 0.9 kg, twins 3.8 ± 0.8 kg, triplets 3.2 ± 0.8 kg, and quadruplets 2.9 ± 0.6 kg. Thus, as litter size increases, dystocia due to oversized lambs decreases (Speijers et al. 2010). Conversely, as litter size increases, the risk of dystocia due to malpresentation of the fetus increases (Fig. 12.11). Male lambs are consistently heavier than females. Smith (1977) noted that the mean weight of male lambs was 4.2 ± 0.3 kg versus 4.0 ± 0.3 kg for female lambs and that this difference was associated with 19% dystocias in male lamb births versus 14% in females.

Various selective breeding methods have been applied to try to reduce the effect of lamb birth weight upon dystocia. Heritability for lamb birth weight is moderate (h^2: 0.38) and is genetically correlated with lamb death risk traits (Everett-Hincks et al. 2014). Thus McSporran et al. (1977) were able to substantially reduce the prevalence of dystocia by breeding from ewes that had lambs of low birth weight and less difficulty at lambing. Unfortunately, there are unfavourable genetic correlations between birth weight and weaning weight and between the risk of dystocia and fleece weight (Brown et al. 2014), both of which could impede genetic selection for lower birth weight.

Because of the benefits upon both dystocia and lamb mortality of achieving lamb birth weights in the range of 4 to 6 kg, the effects of maternal nutrition upon lamb birth weight have been extensively studied. The consensus of these studies is that maternal nutrition has little effect. For example, in a meta-analysis of experiments with New Zealand Romney ewes, Schreurs et al. (2012) concluded that the influence of a ewe that is heavier at Days 0, 50, 100, or 150 of gestation on the birth weight of its lamb was very small. The effect is also more dependent on litter size than upon ewe weight. Likewise, there was neither an effect of dietary restriction during



• **Fig. 12.11** Relationship of dystocia caused by either (A) oversized lambs or (B) malpresented lambs with litter size for different sire breeds: Blackface ●, Swale ●, and Texel ●. (Redrawn from Speijers et al. 2010.)

mid- to late pregnancy (Kenyon et al. 2011) nor an effect of a high plane of nutrition (Corner-Thomas et al. 2015) upon lamb birth weight. It appears that the reason for the failure to find an effect of maternal nutrition is due to the ability of the placenta to compensate under such circumstances. Thus severe undernutrition during early and midgestation reduces the number of placentomes,

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TABLE 12.18 External pelvic dimensions and body weights of mature ewes of different breeds

Breed	Dimension				
	Intertuber Coxal (cm)	Lateral Intertuber Ischial (cm)	Medial Intertuber Ischial (cm)	Rump Length (cm)	Weight (kg)
Unimproved Breeds					
Soay	13.1	11.3	5.6	17.4	21.7
North Ronaldsay	15.1	12.4	6.3	18.6	24.8
Shetland	16.6	12.7	6.4	19.5	34.6
Selected Breeds					
Scottish Blackface	21.0	16.9	9.0	23.4	66.4
Clun Forest X	26.4	16.5	8.8	23.6	61.6
Suffolk	22.1	18.3	8.8	25.5	79.5

From Silva & Noakes (1984)

but they subsequently increase in size and alter their shape (Steyn et al. 2001). Therefore, if nutrient intake is increased in the last trimester, then the placenta is probably more efficient in nutrient transfer and the fetus grows more rapidly.

Parity affects lamb birth weight. Smith (1977) showed that birth weight increases from first (3.5 ± 0.1 kg) to second (4.1 ± 0.1 kg), to third (4.4 ± 0.1 kg) through to fourth (4.5 ± 0.1 kg) parity. Ewe lambs typically produce lighter lambs than do ewes. Corner et al. (2013) showed that birth weight of lambs from single-bearing ewe lambs were lighter (4.4 ± 0.1 kg) than those born of ewes (5.5 ± 0.1 kg).

Pelvic Capacity of the Dam

Assessments of the size of the maternal pelvis have been undertaken to determine the contribution of this factor to fetomaternal disproportion. Fogarty and Thompson (1974) showed that pelvic dimensions of Border Leicester and Dorset Horn ewes that lambed normally were larger than those which had dystocia. External pelvic dimensions were measured in a large number of different breeds, including several rare breeds which had not been subjected to selection pressures for growth traits and carcase quality (Silva & Noakes 1984). Table 12.18 shows the wide variation in pelvic size, with breeds such as the Soay, North Ronaldsay and Shetland having small pelvic dimensions, whereas the Scottish Blackface, Clun Forest, and Suffolk have larger pelvic dimensions. However, when the pelvic dimensions are compared with the body weights of the ewes for the different breeds, it is noticeable that the 'unimproved' breeds have relatively larger pelvic dimensions than the more selected breeds (Table 12.19). Because fetal weight is between 6% and 8% of maternal body weight, then the relatively smaller pelves of those breeds that have been subject to genetic selection to produce large lambs at birth are more likely to have dystocia than the rare breeds that have largely been left to the influences of natural selection.

Pelvic area of Romney ewes, as assessed by radiographic pelvimetry (McSporran & Wyburn 1970, McSporran & Fielden 1979), has been related to their risk of dystocia. Haughey and Grey (1982) and Cloete and Haughey (1990) confirmed that radiometry is a relatively accurate means of estimating the size of the pelvic inlet, whereas others (Bilbe et al. 2005) have used computerised tomography for the same purpose. Attempts to correlate external bodily measurements with internal pelvic dimensions have been shown not to be particularly useful.

TABLE 12.19 Ratio of pelvic dimensions: body weight of adult ewes of different breeds

Breed	Ratio of Medial Intertuber Ischial Dimension to Body Weight	Ratio of Sum of Pelvic Measurements to Body Weight
Unimproved Breeds		
Soay	1.0	1.0
North Ronaldsay	0.98	0.95
Shetland	0.72	0.72
Selected Breeds		
Scottish Blackface	0.53	0.48
Clun Forest X	0.55	0.51
Suffolk	0.43	0.43

From Silva & Noakes (1984)

Faulty Disposition of the Fetus

Presentation and Position

About 99% of foals and 95% of calves are delivered in anterior presentation. When sheep are pregnant with a single lamb, they show a similar percentage of anterior presentations to cattle, but with twins there is a considerable proportion of lambs born in posterior presentation. The polytocous sow, bitch and queen deliver 30% to 40% of fetuses in posterior presentation. In monotocous species posterior presentation is associated with an increase in the risk of dystocia. In the mare, although posterior presentation only accounts for 1% of births, it accounts for approximately 16% of hospital referrals for dystocia (Frazer 2011). In the cow dystocia occurs in approximately 50% of deliveries in uncomplicated (i.e., without hindlimb flexure) posterior presentation (Citek et al. 2011).

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In the ewe uncomplicated posterior presentation was responsible for dystocia in 10% of malpresented lambs, and breech presentation was responsible for dystocia in a further 12% (Dwyer & Bunger 2012). In polytocous species, uncomplicated posterior presentation has only a marginal effect upon birth difficulty, whereas flexion of the hindlimbs causes an increase in the risk of dystocia.

During pregnancy, the fetus assumes a disposition that occupies as little uterine space as possible. However, during parturition, it must assume a disposition that enables it to be expelled through the birth canal. These changes in disposition occur, according to species, before or at the time of labour. Faulty presentation appears to be a failure of the changes of disposition that occur during that time.

Horse. The equine fetus is freely movable in the uterus in the early stages of gestation and is equally likely to be in anterior or posterior presentation until the fifth month. Ginther (1998) described the selection of anterior presentation occurring after that time, due to closure of the uterine horns by the eighth month, thereby confining the fetus to the uterine body. Thus the probability of an anterior presentation increases after the fifth month, such that 98% of foals have adopted an anterior longitudinal presentation by 8½ months gestation (Vandeplassche 1957). Thereafter, the foal normally assumes a dorsopubic position, with the hindlimbs becoming encased in one uterine horn, so that the feet rest on the dorsal surface of the uterus, directed towards the cervix, often pushed into the rectogenital pouch. This 'entrapment' of the feet prevents the fetus from reverting to a posterior presentation (Ginther 1998). At this stage of gestation, the cranial part of the fetus can rotate between dorsoilial and dorsopubic positions (Frazer 2011). During parturition the head and forelimbs become extended, and the cranial portion of the fetus rotates into a dorsoilial position. This appears to be an active process on the part of the foal, possibly in response to increasing uterine tone: a fetus that is dead or nonviable is less likely to make this postural transition so is more likely to result in a dystocia due to malpresentation. Complete rotation of the foal into an extended, dorsosacral position is only completed during second stage labour. Posterior presentation predisposes the mare to dystocia because the rotation and extension of the fetal extremities is impeded; so posterior presentations are commonly in dorsoilial presentation, often with bilateral hip or hock flexion (Frazer 2011).

A small proportion (~0.1%) of foals becomes transverse presentations, in which the extremities of the fetus occupy the uterine horns, while the uterine body is largely empty. This presentation probably arises at about 70 days of gestation, when the uterus normally changes from a transverse to a longitudinal direction in front of the maternal pelvis as a result of the allantochorion passing from the pregnant horn into the uterine body. In the abnormal situation either the allantochorion does not intrude into the uterine body or the major, rather than the normally minor, branch of the allantochorion passes into the nonpregnant horn and is followed by the amnion, containing a fetal extremity. Normally, neither the amnion nor the fetus passes into the nonpregnant horn.

Cow. During the first 2 months of gestation, the calf has no definite polarity, and during the third month there are equal numbers of anterior and posterior presentations (Abusineina 1963). Throughout the fourth, fifth, and first half of the sixth months, the majority of fetuses were in posterior presentation, but during the sixth month the situation began to change so that, at the end of that month, anterior and posterior presentation frequencies were equal. By the middle of the seventh month, the majority of fetuses were in anterior presentation. Beyond the seventh month, posterior presentation was rare and transverse presentation exceedingly rare. However, posterior presentations are more common in bull than heifer calves, suggesting that the greater size of the male calf may interfere with the process of turning into anterior presentation (Holland et al. 1993). During the last week of gestation the fetus remains in a dorsopubic or left dorsolateral position (Dufty 1973), regardless of presentation, but as myometrial contractions start during first stage labour, the calf rotates into an anterior presentation, dorsosacral position, with the head and forelegs extended (Norman & Youngquist 2007). This period of rotation and extension is dependent on fetal movement in response to myometrial contraction: fetal activity is maximal about 3 days before parturition. This phase of activity has been referred to as the 'righting reflexes' (Fraser 1976a). Holland et al. (1993) noted that, if fetal muscles' competence of endurance was not adequate, the righting reflex would not occur and the resulting presentation would be ventral. The maintenance of myometrial activity is a prerequisite for these fetal movements. Jöchle et al. (1972) found that progesterone given to parturient cows caused a high incidence of postural dystocia, which could be due to maintaining the 'progesterone block' on the myometrium (see Chapter 6), thereby reducing the stimulation of the fetal calf to initiate its righting reflexes. It is also well recognised that there are increased frequencies of postural aberrations in premature births, in which uterine inertia is more prevalent, and with twins, in which there is not only an increased likelihood of uterine inertia, but also reduced space, thereby interfering with the ability of the limbs to extend.

The natural forces that bring about these changes in polarity are therefore reflex fetal movements that occur in response to: changes in the intrauterine pressure due to myometrial contractions; movements of adjacent abdominal viscera; or contraction of the abdominal musculature. The preponderance of posterior presentations in early gestation would be the expected result of suspending an inert body with the same centre of gravity as the fetal calf. With the development of the fetal nervous system, and a consequent appreciation of gravity, the fetal calf begins to execute righting reflexes that bring up the head from the dependent part of the uterus. Hence posterior presentation, rather than being regarded as an obstetrical accident, could be caused either by a subnormally developed fetus or by a uterus deficient in tone. In addition, the size of the fetus and uterine space influence the ease with which a fetus can change its polarity *in utero,* so there is a much higher percentage of posterior presentations in bovine twin births, with a higher than average percentage of posterior presentations occurring with excessively large fetuses.

Posture

Holland et al. (1993) noted that, in cattle, after the righting reflex (i.e., once the fetus has adopted an extended, dorsopubic position) a low level of fetal activity is required to maintain an adequate posture. If normal fetal muscular activity does not occur during this period, dystocia due to faulty posture can ensue. Thus Fraser (1976b) found that a reduction in fetal movements during the righting period was a primary cause of fetal malposture. Interestingly, during hypoxia, fetal movements are either markedly diminished or there are erratic limb and head movements (Dufty & Sloss 1977), which Holland et al. (1993) surmised could contribute to the development of abnormalities of posture.

Similar effects pertain to the sheep. Dwyer and Bunger (2012) concluded that malpresentation may occur if a large lamb is forced into an incorrect disposition during its passage through the birth canal or if the fetus is already incorrectly positioned at the onset

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of labour. Grommers et al. (1985) concluded that narrowness of the maternal pelvis was at least as important as fetal oversize to the pathogenesis of dystocia due to shoulder flexion. Lambs with one of both forelimbs retracted were significantly heavier than lambs in other presentations, whereas lambs in a breech or head back presentation were significantly lighter than lambs in other presentations.

Lateral deviation of the head is a postural abnormality that deserves special mention. It may be due to the same factors as those noted previously, but lack of uterine space may be more important, and it may arise during late gestation rather than during birth. A congenital deformity known as wryneck, in which the head and neck are fixed in flexion because of ankylosis of the cervical vertebrae, arises during the peculiar bicornual gestation of the mare (Vandeplassche 1987).

In polytocous species the relatively larger pelvis of the dam and smaller limbs of the fetus mean that the disposition of the fetal limbs is less important than in monotocous species. Thus many piglets, puppies, and kittens are delivered normally with their limbs in postures that would have caused dystocia in the foal and calf. However, if a female of a polytocous species is parturient with an abnormally low number of fetuses, there is likely to be some degree of fetomaternal disproportion and, in these circumstances, malposture of the limbs may cause dystocia. Moreover, the high proportion of flexion of the head/neck of malpresented puppies and kittens suggests that similar effects of hypoxia, fetal movement, and/or constraints of the maternal birth canal are likely to be contributors to the aetiology of such dystocias.

Maternal Dystocia

Dystocia due to maternal causes, including the aetiology of such dystocia, is discussed in Chapter 13.

Consequences of Dystocia

The consequences of dystocia are numerous, ranging from increased stillbirths and perinatal mortality to severe trauma to the offspring or its mother. Dystocia results in:

- Harm to the welfare of the dam,
- Harm to the welfare of the offspring,
- Increased risk of stillbirth or perinatal mortality of the offspring,
- Increased neonatal morbidity,
- Increased mortality rate of the dam,
- Reduced productivity of the dam,
- Reduced subsequent fertility and increased chance of sterility in the dam,
- Increased likelihood of puerperal disease in the dam, and
- Increased likelihood of subsequent culling of the dam.

Cattle

Stillbirth and perinatal mortality are major causes of economic loss in cattle, and both are highly related to dystocia. Thus Paterson et al. (1987) found that 46% of all preweaning deaths could be attributed to dystocia. Sloss and Dufty (1980) showed that about a third of the total of 17% of fetal and calf losses occur at the time of parturition and that most of these arise from calving difficulties. Calves born to cows that suffered from dystocia were five times more likely to die in the immediate perinatal period than those resulting from normal calvings (Azzam et al. 1993). Wittum et al. (1994) reported that 17.5% and 12.4% of calf mortality was associated with dystocia and stillbirth, respectively, and Johanson and Berger (2003) found that perinatal mortality of calves born after dystocia was 2.7 times that for calves born unassisted. Laster and Gregory (1973) found that calves born to dystocic calvings were four times as likely to die as those born from normal calvings. The severity of the dystocia affects the probability of a stillbirth. McGuirk et al. (2007) found that the calf mortality rate in dairy cows was 2.6% after normal calvings, which increased to 9% after slight dystocia, 54% after severe dystocia, and 27% after Caesarean surgery. Tenhagen et al. (2007) found higher rates of stillbirth after mild dystocia (live calves: 78% versus 98%) and severe dystocia (74% versus 92%) than in normal births. Waldner (2014), likewise, associated the risk of stillbirth with the severity of dystocia; in approximately 30,000 calvings in cow–calf herds, the odds ratio for stillbirth was 2.1 for an 'easy pull', 8.5 for a 'hard pull', 22.2 for manipulation, and 11.2 for Caesarean surgery, compared with unassisted calvings. Finally, Villettaz Robichaud et al. (2017) found that early assistance in cases of dystocia was associated with fewer calves being born dead than later assistance (odds ratio versus unassisted calvings: 1.5 and 10.3, respectively).

Culling rates are typically increased and milk yields decreased in cows that suffer dystocia. In a study in Bavaria, Sauerer et al. (1988) reported that the culling rate of cows that suffered from dystocia was increased by 18%, and the calving interval extended by 11–14 days. Milk yield was not affected unless the calf was stillborn, in which case the yields were reduced by 50 kg, 126 kg, and 148 kg for the first, second, and third lactations, respectively. In a large study of approximately 72,000 Holstein cows in the United States, Dematawewa and Berger (1997) showed that differences between cows that had a normal calving and those that had an extremely difficult calving for milk yield, fat yield, protein yield, number of days open, services/conception, and percentage cow deaths were 703.6 kg, 24.1 kg, 20.8 kg, 33 days, 0.2 services, and 4.1%, respectively (Table 12.20). McGuirk et al. (2007) estimated the likelihood of culling or retention in the (dairy) herd after dystocia, showing that cows that had severe dystocia were more likely to die, be culled, or have poor production than those

TABLE 12.20 Effect of dystocia on losses of 305-day milk, fat, and protein yields and on increases in days open, number of services, and cow deaths for cows in all lactations, relative to cows with dystocia score = 1

	Dystocia Score			
Trait	2	3	4	5
Milk (kg)	–74	–165	–193	–704
Fat (kg)	–2.5	–5.7	–6.2	–24.1
Protein (kg)	–2.2	–5.1	–5.7	–20.8
Days open	+7.4	+10.9	+16.5	+32.6
Services per conception	+0.06	+0.07	+0.10	+0.20
Cow death (%)	+0.24	+0.55	+1.48	+4.12

Dystocia scores: 1 = no problem; 2 = slight assistance; 3 = needed assistance; 4 = considerable force needed; and 5 = extreme difficulty
Based on Dematawewa & Berger (1997)

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TABLE 12.21 Percentage of cows experiencing various degrees of dystocia, and their fates

	Cows' Fate (%)			
Severity of Dystocia	Normal	Retained in Herd, but 'Poor'	Poor and Culled	Died
Normal	97.3	2.2	0.2	0.3
Slight	93.7	5.2	0.5	0.6
Severe	61.7	24.7	3.1	10.5
Caesarean surgery	80.0	13.3	6.7	0

Based on McGuirk et al. (2007)

that had normal calvings (Table 12.21). Similarly, Tenhagen et al. (2007) showed increases in culling rate, reductions in conception rate, and increased days from calving to conception according to the severity of dystocia.

Salman et al. (1991) identified dystocia as the disease with the largest veterinary treatment cost in the Colorado National Animal Health Monitoring System. Similarly, Miller and Dorn (1990) found that, in Ohio, 5% of the total cost of disease prevention and occurrence per cow per year was due to dystocia. Kossaibati and Esslemont (1995) estimated that, at that time in the UK, the direct and total costs were £141 and £310, respectively: figures that were influenced by the current commercial value of calves. Dematawewa and Berger (1997) estimated the costs due to dystocia to be $0.00, $50.45, $96.48, $159.82, and $397.61 for no assistance at calving, slight assistance, needed assistance, considerable force needed, and extreme difficulty, respectively. In a UK farm-based survey (1995–2002), involving approximately 10,000 noninduced calvings, the total costs of slight dystocia and severe dystocia were approximately £110 and £350 to £400, respectively (McGuirk et al. 2007). In that study, the major cost was associated with the increased labour required at delivery, the increase in the number of days open and the costs associated with cow and calf mortality, and cow culling.

Sheep

Losses due to dystocia in sheep are largely due to the increased incidence of perinatal lamb mortality. Hence most of the factors that affect the incidence of dystocia in sheep are also associated with the risk of perinatal lamb mortality (Welmer et al. 1983, Woolliams et al. 1983, Speijers et al. 2010). Because lamb vigour and suckling is also impaired in lambs that have been born as the result of an assisted lambing (Dywer & Bunger 2012), much perinatal lamb mortality that is ostensibly due to starvation or hypothermia may well have had an 'ultimate' cause of dystocia. Brown et al. (2014) also noted that most postmortem traits were highly correlated with lambing ease and, fascinatingly, that thoracic circumference of the lamb was related to the risk of death due to mismothering. Finally, Everett-Hincks et al. (2014) found that the direct genetic correlation between lamb birth weight and survival at 3 days postpartum was negative, such that the genetics responsible for heavier birth weight were correlated with lower survival rates, decreased viability at birth, and increased dystocia rates. The effects on fertility will be less apparent because in most production systems ewes have at least 6 months after they have lambed before they are served again. Severe trauma at parturition will result in lesions to the genital tract, which may cause subfertility or sterility. It is much more difficult to quantify other production losses, and evidence is equivocal whether severe dystocia affects postnatal growth rates. The compromises of ewe welfare that are due to dystocia are less equivocal. Scott (2005) noted that poor hygiene, mediocre husbandry, and the decision to slaughter dystocic ewes rather than seek veterinary attention for them result in significant risks to the welfare of ewes during the lambing period. He advocated the routine use of epidural analgesia during manipulative obstetrical procedures and the use of NSAIDs to provide postbirth pain relief. He also encouraged the provision of effective antibiotic treatment regimens for ewes requiring such treatment.

Pig

It is well recognised that in the sow most stillbirths occur intrapartum as a result of dystocia and the delayed expulsion of the litter. There are substantial data on stillbirth rates in sows. It is generally accepted that the rate should not exceed 7% of total piglets born, and if it exceeds 10%, then there is a need for an investigation (Carr 1998). Kirkden et al. (2013) summarised the relationship between stillbirth and dystocia by noting that stillbirth, low piglet viability in the immediate postnatal period, low vitality during the first few days of life, and postnatal mortality are all closely linked. They considered that perinatal asphyxia is the proximate cause in most cases of stillbirth, which also results in reduced viability and vitality, both of which increase the risk of postnatal mortality. Dystocia, in which human intervention is required, is associated with fetal asphyxia and, hence, is a risk factor for stillbirth (see Chapter 17).

Horse

Because of the relatively early separation of the placenta, foal survival is very short: thus if there is dystocia, there is a very high risk of stillbirth. In a survey involving 3527 cases of abortion, stillbirth and perinatal foal mortality in horses, 196 could be directly attributed to dystocia, and dystocia was probably also involved in a substantial number of the other causes of neonatal death. Birth-related trauma was present in 50% of dystocia cases. Dystocia was found to be responsible for 20% of foal mortality within 48 hours of birth (Haas et al. 1996). In a study of an equine referral hospital (Byron et al. 2002), involving 247 dystocias, 91% of the mares survived, but only 42% of the foals were born alive. Even fewer foals (29%) survived to time of discharge. The interval from hospital admission to delivery of the foal had no effect on foal survival rates. However, there was a significant effect on survival rate to discharge and the time interval from rupture of the allantochorion to delivery of the foal for the survivors and the nonsurvivors: 71.7 ± 34.3 and 85.3 ± 37.4 minutes, respectively (Byron et al. 2002). McCue and Ferris (2012) associated the risk of stillbirth or perinatal morbidity or mortality with the duration of second stage labour.

Mares that suffer from severe dystocia should not be served at the foal heat; if they are, pregnancy rates will be lower than normal for this stage and a small number will suffer from traumatic injuries, which may result in sterility.

Dog

Munnich and Kuchenmeister (2009) reported that dystocia was related to the risk of there being hypoxic and dead puppies within litters. They noted that the risk of hypoxic and/or stillborn puppies increased when there was fetal malpresentation, uterine inertia, or

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prolonged parturition. Prolonged labour was associated with an increase in stillbirths, particularly in the pups that were born later in the whelping process. Darvelid and Linde-Forsberg (1994) also noted that very small litter size was associated with uterine inertia, prolonged parturition, and an increase in the risk of dystocia or stillbirth.

Prevention

Fetomaternal Disproportion in Cattle

The following guidelines were proposed by Drew (1986–87) in relation to the breeding of Holstein–Friesian heifers in the UK for the prevention of fetomaternal disproportion.

Management at Service

- Ensure body weight at the time of service is more than 260 kg.
- Take care when selecting the service sire. Most dairy sires now have published estimated breeding values (EBVs) for traits such as gestation length and calving ease
  If breeding by artificial insemination bulls:
  - Select a well-proven bull of high genetic merit.
  - Select a bull that has been used successfully on heifers on several farms or, if this is not possible, one with a lower than average incidence of calving difficulties (low EBV for birth weight) and gestation length when used on cows.

  If breeding by natural service bulls:
  - Avoid bulls of large breeds.
  - Select a bull with a record of easy calvings or, if this is not possible, one that has a sire with a good record.

Management Before Calving

- Adjust feed levels to avoid calving in an overfat condition.
- Restrict energy intake in the last 3 weeks of pregnancy.
- Check iodine and selenium levels if calf mortality has been high in previous years.
- Ensure supplementary magnesium is provided during the transition period.
- Ensure that an adequate exercise area is available.
- Observe the heifers at least four or five times daily during the last 3 weeks of pregnancy, especially if short-gestation-length bulls are used.
- If possible, run a separate heifer group or run the heifers with dry cows. If fed with the milking cows, ensure concentrate feeding is restricted to the amount required to acquaint the heifer with her postcalving diet.

Management at Calving

- Calve grazed heifers in their field or paddock if possible. Housed heifers should calve in familiar surroundings. Avoid moving them to a calving box unless essential for adequate assistance.
- Ensure the field is well fenced to avoid the possibility of heifers rolling into positions in which it is difficult to assist.
- Observe hourly (approximately) when calving starts. Too frequent observations (more frequently than half-hourly) can delay calving.
- Be a good stockperson. Watch for signs of fear, abnormal pain, or distress and be ready to assist if these are noted or if calving is prolonged.
- Ensure that the stockpersons are trained to identify potential problems and know when to call professional help. If calving aids are used, instruction should be given as to the correct method of application.
- Call professional advice if an unusually high percentage of the first heifers to calve require assistance – there may be a herd problem, which will affect the whole group.

In the case of crossbreeding or purebreeding calves for beef production, the same principles apply. Thus:

- With well-grown heifers, when breeding purebred replacements, select sires on their ease-of-calving records (low EBVs for birth weight) and normal (i.e., not unduly long) gestation lengths for the particular breed.
- In crossbreeding for beef production from dairy herds, avoid sires of the larger breeds, such as Simmental and Charolais, for heifer inseminations; instead use a known 'easy calving' Angus or Hereford bull. For second and later parities choose a bull of a larger breed on his ease-of-calving record and gestation length.
- In beef production from beef breeds, for heifer pregnancies use either a sire of a smaller beef breed or a within-breed sire of good ease-of-calving record and gestation length. For later parities use a bull either of the same or larger breed, both based on their calving ease and gestation length.

When applying the previous principles in the production of offspring for beef, whether purebred or crossbred, it should be noted that the weight of the calf at birth, assuming equal gestation length, bears a direct relationship to its weaning weight and to its subsequent slaughter weight, on which the profitability of the enterprise largely depends. On the other hand, unduly large calves at birth predispose to calf deaths and to maternal morbidity, mortality, reduced milk yield, and infertility. Thus a breeder must consider how much increase in birth weight can be tolerated in return for increases in growth rate and weaning weight.

If dystocia due to fetomaternal disproportion is anticipated, gestation can be shortened by the premature induction of calving as described in Chapter 8.

Dystocia in Sheep

Meat and Livestock Australia (2016) recommendations for minimising the risk of dystocia are:

- **Genetics:** Use BVs for lambing ease and lamb birth weight can be used to assist in eliminating lamb birthing difficulties. When looking at BVs for birth weight, avoid both very low and very high birth weights.
- **Measure to manage:** Using tools, such as scanning all mated ewes to identify empties, single, and multiple carrying ewes, can be used to allocate feed most efficiently and help with monitoring lamb and ewe loss at weaning to identify losses. Overfeeding ewes bearing single lambs is associated with an increase in the risk of dystocia in such lambs.
- **Ewe condition:** Achieve a target condition score (CS) of 3 (1 (poorest) to 5 (fattest) scale) for ewes at the start of mating and maintain ewes at that condition score thereafter. If ewes reach a CS of 4, the risk of dystocia occurring increases significantly.
- **Keeping check:** Monitor ewes close to lambing so that timely and correct interventions can be made during birth.

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13

Maternal Dystocia: Causes and Treatment

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Maternal dystocia includes defects of the birth canal and defects of the expulsive forces. The main maternal causes of dystocia are summarised in Fig. 11.1.

Defects of the Birth Canal

Pelvic Constriction

Developmental abnormalities of the pelvis are generally rare in animals. However, the pelvic inlet of the achondroplastic breeds of dog is flattened in the sacropubic dimension, and this, together with the large head of the fetus in brachycephalic breeds, is a common cause of dystocia. The role of inadequate pelvic size in the aetiology of dystocia due to fetomaternal disproportion is discussed in Chapter 13, and the management of such dystocias is discussed in Chapter 13.

Pelvic constriction after fractures, in which there has been poor alignment of the pelvic bones, can cause dystocia in any species. It is in those that are particularly prone to road traffic accidents, such as dogs and cats, that the frequency is highest. For this reason it is good preventive medical practice to ensure that any bitch or queen cat that has suffered from such an injury is radiographed before breeding, to ensure that the pelvic canal is capable of allowing a normal fetus to pass through at parturition without obstruction. In cattle, sacroiliac luxation can occur as a result of and as a cause of dystocia. It occurs during calving, probably as a result of marked relaxation of the pelvic ligaments and overexertion in rising after parturition. In severe cases the sacral area appears to be dropped, and both tuber coxae are very pronounced (Fig. 13.1). The reduction in crosssectional area of the pelvis will result in dystocia if the animal survives to a further calving.

Failure of Cervical Dilation

The cervix provides an important protective physical barrier for the uterus during pregnancy. Several days before and during the first stage of parturition, the cervix undergoes considerable changes in its structure so that it can dilate sufficiently to allow the passage of the fetus (see Chapter 6). Incomplete dilation occurs in cattle, goats, and sheep; in the latter species it is one of the more common causes of dystocia. The degree of incompleteness of cervical dilation varies from virtually complete closure to the situation in which there is just a small rim of cervical tissue present, which is sufficient to reduce the size of the birth canal and cause obstruction. The fact that it is a disorder of the ruminant cervix perhaps suggests a common aetiology because in all three common domestic species, the cervix is a tough fibrous structure with substantial amounts of collagen.

Cattle

In cattle incomplete dilation of the vulva or cervix may occur in both the heifer and the pluriparous cow. Mee (2008) noted that incomplete dilation of the vulva is more common in primiparous animals whereas incomplete dilation of the cervix is more common in pluriparous cows. There are a number of factors that contribute to the condition, of which failure of the hormonal mechanisms that cause the change in structure of cervical collagen during the early stages of labour is probably the most important (see Chapter 6). This, in turn, may be related to prepartum oestrogen concentrations and/or the effects of stress upon the birth process. Inadequate precalving oestrogen concentrations and a raised cortisol to progesterone ratio can result in impaired relaxation of the cervix and pelvic ligaments. These endocrine disturbances can, in turn, be the result of environmental factors, such as the continuous presence of an observer, confinement, or overcrowded calving accommodation. As a consequence, there is reduced uterine motility, cervical dilation, and abdominal contractions with resultant prolonged calving and dystocia (Mee 2008). Also, weak uterine contractions, which would be relatively ineffectual in causing dilation of the ripened cervix, are probably involved in the pathogenesis. In the pluriparous cow, hypocalcaemia (which may be subclinical) can impair uterine contractions and perhaps prevent cervical dilation. Likewise, failure of the fetus to engage in the cervix (such as can occur with a breech presentation or simultaneous presentation of twins) and uterine torsion can also be a cause of failure of cervical dilation. It is, however, important to recognise that premature intervention in a calving can lead to an incorrect presumption of failure of cervical dilation (i.e., if first stage labour is not fully completed at the time of intervention), which, in turn, can lead to iatrogenic damage to the cervix. Finally, it should be remembered that the period of time over which the cervix is dilated is relatively short. If the calf is not delivered during that time window, the cervix will start to close again, trapping the fetus within the uterus.

The characteristic signs of discomfort associated with the first stage of parturition are commonly only mild and transient, so that often it is difficult to ascertain accurately for how long labour has been in progress. On vaginal examination the degree of dilation

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• **Fig. 13.1** Sacroiliac luxation in a cow. The sacral areas appear to be dropped and the tuber coxae are excessively pronounced. (Courtesy Dr. Jakob Malmo.)

of the cervix varies from a lumen that is just a few centimetres in diameter through to a rim of cervical tissue about 5 cm broad separating the vagina from the uterus. In the latter case it is common for the amniotic sac to have passed through the cervix and be present at the vulva; it may also have ruptured with escape of amniotic fluid. Sometimes fetal limbs have passed into the cranial vagina. At this stage it is advisable to determine whether the cow is showing signs of hypocalcaemia and, if so, to correct the calcium deficiency, either by giving calcium orally or parenterally. Whether to wait for the cervix to dilate (i.e., assuming that the cow has not completed first stage labour), whether to attempt vaginal delivery, or whether to proceed to Caesarean surgery will depend on the extent to which there is failure of cervical dilation, the viability of the fetus, and an assessment of whether the cervix will complete dilation in response to gentle, sustained traction. Premature delivery by traction is likely to result in trauma to the cervix, reduced calf viability, and downer cows; however, waiting too long also results in impaired calf viability. It is probably sensible to leave the cow for a maximum of 2 hours and then, if there has been no progress in parturition, the alternative option should be followed (Mee 2004). In cases of abortion the cervix often fails to dilate properly and the fetus may be retained, subsequently to undergo putrefactive maceration in the uterus.

Sheep and Goats

Incomplete dilation of the cervix of the ewe and doe goat is descriptively named 'ringwomb'. It accounts for a substantial number of the ovine dystocia cases referred to veterinarians. It occurs most commonly in 3-year-old ewes and is commonly associated with prolonged gestation (Kerr & Dailey 1999). The aetiology of the condition appears to be multifactorial: Purohit (2006) identified hypocalcaemia, hypophosphataemia, trace element deficiencies, uterine inertia, and failure of the fetus to engage the cervix as predisposing causes, although Kerr and Dailey (1999) considered that none of these factors was directly causative. Many cases of ringwomb follow preparturient prolapse of the vagina (see Chapter 10), and both conditions occur in similar circumstances of breed and environment. The role of oestrogens in the condition is less clear than in the cow, as progesterone concentrations are relatively higher and oestrogen concentrations relatively lower in the ewe at parturition than in the cow (Klauke & Hoffmann 1992). Moreover, oestradiol does not cause dilation of the nonpregnant ovine cervix (Masoudi et al. 2014), although oestradiol in combination with high concentrations of oxytocin may do so (Masoudi et al. 2012). On the other hand, ringwomb has been induced experimentally by stilboestrol administration, so Hindson and Turner (1972) suggested that ringwomb might be caused by ingestion of oestrogenic substances by pregnant sheep. A mechanism that has been postulated to explain these effects is that alterations of the oestrogen to progesterone ratio affect intracervical synthesis of prostaglandins E and F and, because these regulate the collagen degradation and remodelling of the collagen matrix that occurs during first stage labour (Challis & Lye 1994), cause failure of cervical ripening. The continued ineffective uterine contractions may cause separation of the placenta from the cotyledons, which can result in death or loss of viability of the lamb (Kerr & Dailey 1999). As in the cow, dystocia can result in a previously dilated cervix closing again.

The condition is suspected when, after protracted restlessness, the ewe does not progress to the second stage of labour. Manual exploration of the birth canal reveals that the cervix is a tight, unyielding ring that will admit only one or two fingers. If it is possible to feel the intact allantochorion beyond the cervix, it is likely that the ewe has not yet completed first stage labour. If the membrane has ruptured and a portion of it has passed into the vagina, the condition is undoubtedly ringwomb (Winter & Hill 2003). If there is a fetid vaginal discharge and necrotic fetal membrane in the vagina, in the presence of a nondilated cervix, there is no doubt that the condition is abnormal and may be due to fetal retention after a failed abortion or dystocia due to some other reason in which the lamb(s) were not expelled after the cervix had dilated normally. When there is doubt over the diagnosis, the ewe should be left for 2 hours and then reexamined to ascertain if any further cervical dilation has occurred, as in normal first stage labour.

There are three main options for treating ringwomb: manual dilation, pharmacological dilation, or Caesarean surgery. Manual dilation is commonly the first recourse to manage cases of ringwomb. Winter and Hill (2003) describe the process of manual dilation, with the insertion of one or more well-lubricated fingers into the cervix and patiently massaging the cervix over 20 to 30 minutes until it relaxes. They emphasise the risks of causing trauma to the cervix by careless or overforceful attempts at manual dilation. Moreover, Caufield (1960) found that only about 20% of cases of cervical dilation failure recognised by him opened naturally and even these required some further assistance to lamb, whereas Menzies (2007) considered that cases of 'ringwomb' that respond to manual dilation are unlikely to represent a true failure of cervical softening.

Various spasmolytics and uterine muscle relaxants have been used in attempts to treat ringwomb. These have largely been unsuccessful, principally as none of these agents act upon the cervix itself. More success has been achieved by combinations of oestrogen, prostaglandin E, and oxytocin. Prostaglandin E can be used to dilate the ovine cervix for AI (Barbas et al. 2013), and there are some reports of its successful use in cases of ringwomb (Azawi et al. 2012, Menzies 2007). However, the method does not yet appear to be sufficiently successful to have become widely used.

Failure to adequately dilate the cervix by manual or pharmacological methods requires that the lambs are delivered surgically. The method of vaginal hysterotomy, whereby the cervix is retracted with vulsellum forceps and then incised by shallow cuts 'at the points of the compass' has been advocated in the past, but is now largely

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regarded as unacceptable on welfare grounds. Hence the preferred method for delivering ewes with ringwomb is Caesarean surgery.

Incomplete Relaxation of the Caudal Vagina and Vulva

This is a relatively common finding in dairy heifers. It seems to be associated with heifers that are in overfat body condition and in herds in which the animals have been moved just before calving, or in which the process of calving has been interrupted by too frequent observations or interventions (Mee 2008).

Treatment requires the patient application of slow and gentle traction. If excessive force is used because of impatience, then there will be perineal damage, which may be so severe that a third-degree perineal laceration will occur (see Chapter 20). If continuous progress is made, then delivery can be affected. Another approach is by inserting both well-lubricated arms (with hands clasped) into the vagina and progressively stretching the vulva and dorsal vagina 'at the 10 and 2 o'clock positions', using the bulk of the operator's biceps. This procedure should be carried out for 5 to 10 minutes. If the vulva will not dilate properly then an episiotomy should be carried out (see Chapter 14). If there is any doubt about the likelihood of success with continuing attempts at vaginal delivery, Caesarean surgery should be performed. There are occasions when large numbers of heifers in a group will be affected. The reason for this is not known; however, if a substantial number are affected, treatment with clenbuterol at the first signs of the onset of the first stage of parturition will delay calving and allow the heifer extra time for the vulva, vagina, and perineum to soften and relax, thus reducing the chances of dystocia.

Leidl et al. (1993) reported that 3% of the dystocias in mares referred to the obstetrics clinic of Munich Veterinary School were due to incomplete dilation of the birth canal; these were all associated with what was described as premature delivery (abortion).

Vaginal Cystocele

Vaginal cystocele is the name given to a condition occasionally encountered in the parturient mare and cow in which the urinary bladder lies in the vagina or vulva. It is of two types:

- Prolapse of the bladder through the urethra. This is more likely to occur in the mare due to the large diameter and distensible nature of the urethra, and the force of the straining efforts in this species. The everted organ will occupy the vulva and will be visible between the labia. The visible surface of the bladder is its mucosa, resulting in a red, 'velvety' appearance.
- Protrusion of the bladder through a rupture of the vaginal floor. When this occurs, the bladder will lie in the vagina or hang down from the vulva. A tear will be evident on palpation, and the outer surface of the misplaced bladder will be its serous coat (Fig. 13.2).

It is important to differentiate both of these types from protrusion of fetal membranes; this is particularly the case in the mare, in which the prolapsed and everted bladder and the velvety (villous) surface of the allantochorion are very similar ('red bag'). In both conditions, the first aim of treatment is to overcome straining; this is best achieved by the induction of epidural anaesthesia with or without sedation. This must be followed by retropulsion of those parts of the fetus that already occupy the vagina. In the case of the prolapsed/everted bladder, it is then necessary to invert the organ again by careful manipulation. When there is a protruded bladder, it must be replaced through the tear in the vaginal wall and the latter sutured. In the mare, if the tear is large, the procedure is best done under general anaesthesia. The fetus should then be delivered by traction after the correction of any faulty disposition.

• **Fig. 13.2** Prolapse of the bladder through a rupture in the floor of the vagina of a parturient cow. The presenting surface of the bladder is serosal, rather than the 'velvety' appearance when the organ is prolapsed/everted through the urethra. (Courtesy Dr. Jakob Malmo.)

Neoplasms

Neoplasms of the vulva and vagina may occur in all species and thus serve as potential causes of dystocia because of physical obstruction, although in fact they seldom do so. In the cow, papilloma, sarcoma, carcinoma (Fig. 13.3), and submucosal fibroma of the vagina and vulva occur, whereas in the bitch the vaginal submucous myxofibroma is common.

Neoplasms of the cervix are so rare in livestock as to be of no consequence in a consideration of the causes of dystocia.

Pelvic Obstruction by the Distended Urinary Bladder

Jackson (1973) described a type of porcine dystocia in which the birth canal was obstructed by the distended urinary bladder being forced back by straining in the form of a mound under the vaginal floor, where it acted like a ball-valve in the birth canal; it was associated with a very relaxed birth canal. Schulz and Bostedt (1995) reported bladder flexion and vaginal prolapse as the third most common cause of dystocia in sows in their survey in Germany. Bladder flexion, which is probably caused by straining, will cause kinking of the urethra, resulting in urinary obstruction and distention of the bladder. Careful catheterisation of the bladder relieves the condition, ensuring that the catheter is not forced through the urethral wall at the point where it is bent.

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• **Fig. 13.3** Squamous cell carcinoma of the vulva of a cow. (Courtesy Dr. Jakob Malmo.)

• **Fig. 13.4** Double external ostia of the cervix of a cow. There was a single internal os, with a bifurcation of the cervical canal at its midpoint.

Other Physical Abnormalities Causing Obstruction

Remnants of the paramesonephric ducts often persist in the cranial vagina of cattle. They generally have the form of one or more 'bands' passing from the roof to the floor just caudal to the cervix and are usually broken during parturition. Sometimes the fetus may pass to one side of them. Occasionally, however, a remnant is of such size and strength that it forms an effective barrier to the birth of the fetus. For example, the cervix may have a single internal os, but the canal then bifurcates, so that the cervix has two external ostia (Fig. 13.4). If both of the forelimbs pass on either side of it dystocia is unlikely to occur. It is important that the obstetrician recognises the abnormality that is present and does not confuse the condition with a partially dilated cervix. To examine the vagina satisfactorily, it is often an advantage to induce caudal epidural anaesthesia and repel the fetus into the uterus. Simple bands of tissue that cause the obstruction can be cut without risk, using a hook-knife or a guarded fetotomy knife of the Colin or Roberts type, but more substantial abnormalities may require Caesarean surgery.

Cases of bifid (septate) uterus and double cervix are occasionally seen on random postmortem examinations of bovine genitalia, and there is generally plentiful evidence that the animals involved have had one or more calves. The condition is surprisingly rarely associated with dystocia, although there are occasions in which both canals had dilated and one forelimb had passed into one canal and the head and the second forelimb had entered the other.

Torsion of the Uterus

Torsion of the uterus is a cause of dystocia in all domestic species. It is most common in buffaloes, relatively common in cattle, and relatively rare in other domestic animals. It is much less common in *Bos indicus* than in *Bos taurus* (Sloss & Dufty 1980). Differences in prevalence between species are considered to reflect differences in the mesenteric suspension and the musculature of the uterus. In the sow, bitch, and queen, torsion is usually of either a single uterine horn, or part of a horn, whereas in other species, the entire uterus rotates along its longitudinal axis.

Cattle and Buffaloes

The prevalence of uterine torsion in cattle is around 0.25% of all births (Lyons et al. 2013), between 3% and 7% of cases of dystocia (Frazer et al. 1996) and between 15% and 25% of cases referred to a veterinary hospital (Pearson 1971). Torsion of the uterus is more common in buffaloes than cattle, with up to 80% of dystocia cases presented to veterinary hospitals being due to this particular cause. Purohit et al. (2011) consider uterine torsion to be the most common cause of dystocia in dairy buffaloes. The aetiology and management of torsion is similar for cattle and buffaloes and so will be considered together.

Aetiology

The aetiology of uterine torsion has been extensively reviewed by Ghuman (2010). Uterine torsion is a complication of late first stage or early second stage labour, although nearly all cases occur during first stage labour. Occurrences in midgestation, during second stage labour and the postpartum period have been described but are rare. There are a number of predisposing factors associated with the development of uterine torsion, but the main causal factors appear to be associated with the anatomy of the uterine attachments and the development of the uterus during late gestation. The bovine uterus is predisposed to torsion because of: (i) the attachment of the broad ligament along the lesser (ventral) curvature of the uterus, which thereby leaves the greater (dorsal) curvature free; (ii) the subilial attachment of the broad ligaments; and (iii) the relatively small increase in the length of the broad ligament of the pregnant horn in late gestation, compared with the massive increase of the pregnant horn beyond its area of attachment. As a consequence, (iv) the uterine horns are not fixed by the broad ligaments but are free within the abdominal cavity, and (v) the pregnant horn comes to lie between the rumen, intestines, and abdominal wall, where it is inherently unstable. Thus anything which causes the uterus to become more mobile is associated with increased risk of torsion (Frazer et al. 1996, Ghuman 2010). By this hypothesis the lower prevalence in *B. indicus* than *B. taurus* cattle can be

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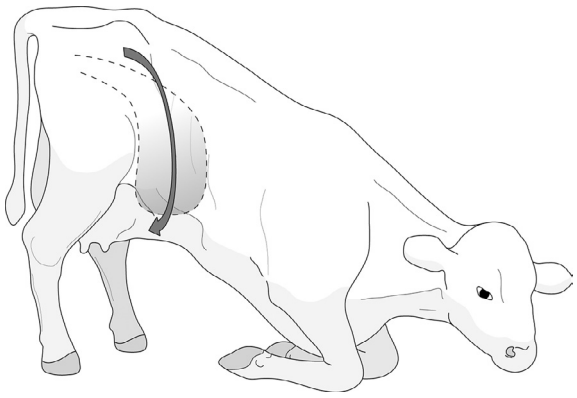

• **Fig. 13.5** A cow in the process of transition between lying and standing. Note that she is resting on her knees (carpal joints) while the hind legs are fully extended, so that the longitudinal axis *(arrowed)* of the gravid uterus of the cow is almost vertical at this stage.

explained in terms of the attachment of broad ligaments on the dorsal side of the cranial portion of the uterus and the ventrolateral side of its caudal portion in *B. indicus,* thereby making it more stable than in *B. taurus.* The higher prevalence in buffaloes can be attributed to longer ligaments and a lack of muscular tone in the broad ligaments of buffaloes compared with cattle (Ghosh et al. 2013).

Predisposing factors are therefore those which cause increased mobility of the bovine uterus at the time when it is inherently unstable. These include, first, factors associated with increased abdominal capacity such as decreased rumen fill (particularly in concentrate-fed cattle; Frazer et al. 1996) and the depth of the abdominal cavity. Second, factors, such as hilly terrain, slipping, sudden movement of the dam, an unsteady walk, and repeated lying and rising, may also predispose to the condition (Ghosh et al. 2013). Third, movement of the uterus about its longitudinal axis may occur when the cow is attempting to rise to her feet from sternal recumbency, particularly when she is in a confined space. She first flexes her forelimbs, so that she bears most of her weight on both knees (carpal joints). This is followed by a forward lurching movement of the head, neck, and the rest of her body, so that both hind legs can be extended; she is now resting on her knees and hind feet. At this stage she may well rest temporarily before making the final effort to extend the flexed carpal joints and stand on all four feet. When the cow is bearing all of her weight on her knees and fully extended hindlimbs, the longitudinal axis of the uterus will be almost vertical, thus allowing it to rotate quite easily about this axis, especially when violent fetal movements occur at this stage (Fig. 13.5). Finally, the violent fetal movements that occur in response to the increasing frequency and amplitude of uterine contractions during the first stage of parturition, as the fetus assumes the normal disposition for normal birth (see Chapter 6), may be the final precipitating factor. Increased birth weight may increase the risk of fetal torsion, but torsion is not associated with fetomaternal disproportion (Aubry et al. 2008). A low ratio of fetal fluid to fetal size may also predispose to torsion (Schonfelder & Sobiraj 2005). Whether parity is associated with the prevalence of torsion is unclear; Ghosh et al. (2013) considered that pluriparous animals are at greater risk due to a decrease in uterine and mesometrial tone but others have not found any clear trend in the risk with age. In the series of cases reported by Frazer et al. (1996), the Brown Swiss breed was overrepresented, whereas the Hereford, Angus, and Jersey breeds were underrepresented compared with Holstein–Friesians. The presence of bicornual twins would appear to stabilise the parturient uterus, and this view is supported by the great rarity of uterine torsion in cases of twin pregnancy.

Clinical Features

Torsion in an anticlockwise direction (as viewed from behind the cow) is more common than in the clockwise direction, accounting for between 50% and 85% of cases (Manning et al. 1982, Frazer et al. 1996, Lyons et al. 2013). Although the uterus rotates about its longitudinal axis, the actual twist in the majority of cases involves the cranial part of the vagina; in the minority of cases in which the twist affects the caudal part of the uterus, there is minimal distortion of the vaginal walls. In the survey by Frazer et al. (1996) 34% of torsions were precervical and did not involve the vagina. In *B. indicus* more torsions are precervical than vaginal (Prabhakar et al. 1994). The most common degree of torsion is either between 90 degrees and 180 degrees (Wright 1958) or 180 degrees and 270 degrees (Pearson 1971, Frazer et al. 1996), perhaps reflecting the difference in severity of cases seen in the field versus those referred to veterinary hospitals. Williams (1943) noted that torsion of >45 degrees is sufficient to result in dystocia and considered that some dystocias attributed to abnormal fetal position (dorsoilial and dorsopubic) could actually be uterine torsions of low magnitude.

The most constant feature of uterine torsion is its association with parturition; Frazer et al. (1996) reported that 81% of their cases were at term. It is generally believed to occur during the first stage of labour because, immediately after it has been corrected, the cervix will be found to be dilated to a variable degree. However, if after correction the cervix is fully dilated or if before correction the membranes have ruptured and portions of them or the fetus are protruding through the cervix, the inference should be that the torsion occurred during early second stage labour. Roberts (1972) believed that torsions <180 degrees cause little interference with gestation and that such torsion, which often arises during advanced pregnancy and may persist for weeks or months, may resolve spontaneously or may be recognised when they cause dystocia at term. The severity of the torsion does not directly affect the survival of the fetus, fetal death being caused by loss of fetal fluids, separation of the placenta, and/or hypoxia due to construction of the uterine circulation.

Signs

Up to the onset of parturition the animal will have been normal, and when it enters the first stage of labour, the usual signs of restlessness due to subacute abdominal pain associated with myometrial contractions and cervical dilation are shown. By far the most common sign is a failure of normal progression of labour (Lyons & Gordon 2013), and the period of restlessness is either abnormally protracted or it wanes and does not progress into second stage labour. If the torsion does not occur until early second stage labour, then a short period of straining will have followed the restlessness but will have ceased abruptly. Beyond that the signs are rather vague; typically restlessness, straining, reduced feed intake, raised tail head, depression, and teeth grinding. Lyons and Gordon (2013) also noted displacement of the vulval commissure in some cases. In severe cases of torsion there may be increasing restlessness, but more probably all parturient behaviour will cease and, unless the animal has been closely observed, there may be no knowledge that parturition has begun. In the study by Frazer et al. (1996), there was pyrexia (23%),

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tachycardia (93%), tachypnoea (84%), straining (23%), anorexia (18%), and a vaginal discharge (13%).

If the condition is not corrected, the placenta will separate, and the fetus will die. Persistent low-grade abdominal pain, progressive anorexia, and constipation will develop. Eventually, the fetus will start to decompose and become emphysematous. Uterine tissues become compromised as a result of the hypoxia that results from compression of the uterine circulation: oedema accumulates in the wall of the uterus, after which degeneration of the myometrial fibres can occur. As a consequence, the uterus or vagina can rupture, major blood vessels of the uterus or broad ligament can rupture, adhesions to surrounding tissue, and/or necrosis of the cervix can occur.

It is also increasingly clear that there is a significant systemic reaction to uterine torsion. This can include loss of tissue fluid and electrolytes due to oedema and transudation and impairment of liver and renal function. Concentrations of haptoglobin, an acute phase protein, increase, which can be regarded as a good indicator of the degree of uterine tissue impairment (Schonfelder et al. 2007). Similarly, blood lactate concentrations may be an indicator of uterine tissue necrosis: Murakami et al. (2017) found that concentrations in cows with uterine necrosis (15 mmol/L) were much higher than in those without necrosis (3 mmol/L). Ghuman (2010) suggested that fluid therapy, plasma expanders (Dextran 40), and correction of acid/base imbalances materially improve the prognosis for cows with more serious/long-standing cases of uterine torsion. However, the use of plasma expanders is likely to be cost prohibitive in cattle practice.

Diagnosis

In cases involving the cranial vagina, diagnosis is readily made by vaginal examination of the cranial vagina, where spiral folds can be palpated. The direction of the spirals indicates the direction of the uterine rotation. The cervix may not be immediately palpable but, by carefully following the folds into the narrowing vagina, the fingers can usually be pressed forwards into the partially dilated cervix. Palpation of the cervix is difficult if the torsion is >180 degrees. When doubt exists about the diagnosis or when the site of the torsion is precervical, diagnosis is assisted by palpating the broad ligament *per rectum.* With the more usual anticlockwise presentation (viewed from behind), the broad ligament can be felt as a tight band running dorsally from the right side, ventrally to the left (Lyons & Gordon 2013). In torsions of less than 180 degrees, portions of the fetus may enter the vagina and the dystocia may be wrongly ascribed to faulty fetal position (lateral or ventral).

Treatment

Rotation of the fetus *per vaginam*. Many cases of uterine torsion can be corrected manually *per vaginam.* The aim of this method is to reach the fetus by insertion of the hand through the constriction of the cranial vagina and partially dilated cervix, and then to apply a rotational force to the uterus via the fetus. A prerequisite for attempting rotation *per vaginam* is that enough of the fetus can be felt to allow manipulation – ideally being able to palpate the head and neck. Success is also much more likely if the cow is standing. With a hand placed flat on the calf's neck, the calf is rocked back and forth while an assistant may apply external force to the abdomen to assist with the rocking of the calf. Once sufficient momentum has been achieved, strong force is applied to rotate the calf in a clockwise direction (in the case of the more common anticlockwise direction of torsion) (or vice versa for clockwise torsions). Once the calf starts to rotate, the operator's hand and arm are quickly turned around and the calf pushed up and over in the uterus in the direction opposite to that of the torsion, using the palm of the hand. Cases of <90 degrees of torsion are generally simple to resolve by vaginal rotation, and cases that are <180 degrees can usually be resolved. Torsions that are >180 degrees become progressively more difficult to resolve by vaginal rotation, as the degree of torsion increases. Long-standing torsions, in which adhesions have formed between the uterus and other abdominal viscera, are often resistant to resolution by vaginal rotation. Administration of clenbuterol before attempting manipulation appears to improve the probability of successful detorsion, although its efficacy is reduced in long-standing torsions or torsions of ≥360 degrees (Menard 1994). It appears to be helpful to have the rear of the cow higher than the front, and epidural anaesthesia may also help. If the calf is alive, the chances of successful detorsion are increased, as its spontaneous movements can aid correction.

• **Fig. 13.6** Use of a detorsion rod to correct a uterine torsion in the cow. Each forelimb of the fetus is attached by snares above each of the fetlocks to the end of the rod. Rotational pressure applied through the handle successfully relieves many cases of uterine torsion. (Photograph courtesy Dr. Berthold Hesselink, Herberex, The Netherlands.)

Detorsion rods (Fig. 13.6) can be used as an alternative to manual rolling *per vaginam.* Snares are attached above each of the calf's fetlocks, then to the end of the rod. A broad handle allows a greater degree of rotational pressure to be applied to the calf than can be achieved by manual effort alone. The use of detorsion rods carries an increased risk of damage to either the uterus or the calf's limbs. Torsion of the uterus cranial to the cervix cannot be treated by vaginal manipulation nor can the rare cases of twists of 720 degrees or more.

Rotation of the Cow's Body: Correction by 'Rolling'. Rolling is indicated if the dam is recumbent, if the fetus is not approachable due to the severity of torsion, if the calf is obviously dead, if the torsion has occurred before the expected time of parturition, or if attempts at detorsion by vaginal manipulation have failed (Ghuman 2010). Rolling methods require an absolute minimum of two competent assistants, although three are preferable (Lyons & Gordon 2013).

The aim is to rotate the cow's body in the direction of the torsion while the uterus remains relatively steady. The cow is cast by Reuff's method on the side to which the torsion is directed. Thus for an anticlockwise torsion the cow is cast onto its left side, and for a clockwise torsion it is cast onto its right side. One assistant holds down the head while the hind and, preferably also the fore, feet are hobbled and attached to ropes. At a given signal

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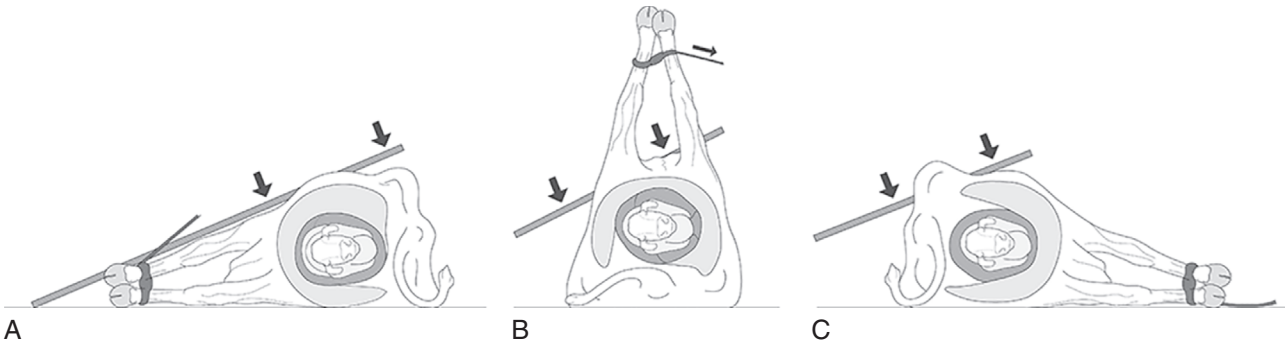

• **Fig. 13.7** Schäfer's method for the correction of uterine torsion in the cow. The cow is cast into lateral recumbency and onto the same side as the direction of the torsion (right side for a clockwise torsion). (A) A plank, 3 to 4 m long and 20 to 30 cm wide, is placed on the flank over the abdomen. An assistant stands on the plank *(black arrows)*. (B) The cow is rolled over slowly to the right in the direction of the torsion. (C) Rolling is completed and the uterine torsion corrected. (Redrawn from Roberts and Hillman (1973).)

a sudden smart coordinated pull is made on the leg ropes so that the cow is rapidly turned over from one side to the other. A vaginal examination is then made to ascertain whether correction has occurred, in which case there is ready manual access to the cervix and probably to the fetus in the uterus, and there may be a flow of fetal fluids from the vagina. If no correction has occurred, the cow is slowly restored to her original position or the legs can be flexed under her body, and she can be turned 180 degrees over her legs on to the original side. The same procedure of rapid turning is then repeated.

Additionally, if the calf is accessible *per vaginam,* the veterinarian may try to better fix the position of the uterus by holding onto the calf while the cow is being rotated. However, Lyons and Gordon (2013) warn of the risk of injury to the veterinarian if this is attempted.

A modification of the foregoing traditional technique described by Schäfer (1946) entails the placing of a wide plank of wood or ladder, 3 to 4 m long and 20 to 30 cm wide, on the flank of the cow, such that one end extends over the paralumbar fossa and the other end rests on the ground. An assistant stands on the plank while the cow is slowly turned over by pulling on the leg ropes (Fig. 13.7). The advantages of this technique are that the plank fixes the uterus while the cow's body is turned and, because the cow is turned slowly, less assistance is required, and it is easier for the veterinarian to check the correct direction of the rolling by vaginal palpation. Moreover, the first rolling is usually successful. Nanda et al. (1991) suggested that, if the torsion is not resolved by three rolls, attempts to relieve it should be abandoned, and Caesarean surgery is indicated. Injury to the cow is more likely if more than three rolls are attempted, with buffalo cows being particularly susceptible.

Delivery After Detorsion. The majority of calves can be delivered *per vaginam* after detorsion of the uterus, either without further obstetrical assistance or after further manipulation and traction. However, the cervix is incompletely dilated in a significant proportion of cows after detorsion: 33% to 35% (Frazer et al. 1996, Aubry et al. 2008, Lyons et al. 2013) of cows that had had uterine torsion successfully corrected subsequently had incomplete cervical dilation. Some of these cows required delivery of the fetus by Caesarean surgery, but in some the cervix later dilated sufficiently to allow vaginal delivery. Lyons and Gordon (2013) noted that opinions differ on how long to leave the cow after correction before further intervention is required. If possible, the calf should be delivered immediately after correction, but when this is not possible, they recommended leaving the cow for up to 3 hours to allow second stage parturition to proceed. However, in cases in which the calf is dead, the cervix is unlikely to dilate further after the torsion has been corrected.

Surgical Correction. If the case cannot be corrected by either of the previous methods or if it is likely that the torsion has been present for longer than 72 hours, a laparotomy should be performed on the standing cow through the left or right flank and an attempt made to rotate the uterus by intraabdominal manipulation. Because a Caesarean hysterotomy may also be required before the torsion can be corrected – or after the torsion is corrected when the cervix will not dilate – a left flank approach is by far the most preferable (see Chapter 16). However, it should be remembered that in cases of uterine torsion there are often loops of small intestine displaced on the left side of the abdomen and that, owing to oedema of its walls, the uterus is unusually friable, and there will be copious amounts of peritoneal transudate in the abdominal cavity. Under paravertebral or field infiltration anaesthesia, a 15 to 20 cm incision is made in the left paralumbar fossa. A hand is inserted, the uterus located, and the direction of torsion confirmed. For an anticlockwise torsion, the hand is passed down between the uterus and the left flank, and a fetal handhold is sought; then an attempt is made to 'rock' the uterus and then rotate it by strongly lifting and pushing over to the right. For a clockwise twist, the hand is passed over and down between the uterus and the right flank and, as before, a swinging manoeuvre is followed by pulling upwards and over to the left. In cases in which it is impossible to rotate the uterus by abdominal manipulation, the fetus must be removed before the torsion can be corrected. The risk of opening the uterus before delivering the fetus is that, once it is removed, the position of the uterus may spontaneously correct itself, leaving the incision in the uterus inaccessible for closure. Otherwise, the uterus is closed, and the torsion resolved subsequently. In many cases, it is not possible to correct the torsion as described previously, as the twisted uterus will be grossly distended, with the uterine wall being oedematous and extremely friable. Attempting to obtain a fetal handhold carries the significant risk of uterine perforation. Therefore it may be best to carry out a laparotomy, using the lateral oblique approach, and subsequently rotate the uterus as is described in Chapter 16. This particular approach provides improved access to the gravid uterus compared with the commonly used left paralumbar fossa approach. It is further advised, once the torsion has been corrected, to allow some time to pass before incising the uterus and removing the calf, in the hope that some of the built-up oedema in the uterine

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wall will dissolve. Great care should be taken when suturing the friable uterine incision, making sure that a good seal is obtained.

Clenbuterol administration facilitates detorsion of the uterus and its exteriorisation for incision and closure (Vermunt 1987). Schonfelder and Hasenclever (2005) calculated the relative costs of euthanasia of cows with irreducible torsions versus the cost of Caesarean surgery and determined that it was far more cost effective to attempt Caesarean than to euthanise such cows.

Sequelae of Torsion. There are records of spontaneous recoveries, but it is generally believed that uncorrected uterine torsion will progress to fetal death, putrefaction, and fatal maternal toxaemia. Fetal maceration with maternal survival is possible. With the adoption of prompt treatment, the prognosis is favourable for mother and fetus, but delay leads to a progressive worsening of the prognosis for the dam (Prabhakar et al. 1994). Roberts (1972) recorded 4.3% maternal mortality. In Pearson's (1971) series of 168 more severe cases treated in a veterinary hospital, only 67 calves were born alive; however, it is certain that a better rate of survival is achieved in the less severe cases treated more promptly on farms. In the series by Frazer et al. (1996), cow survival was 78%, and calf survival was 24%.

Sheep and Goats

The frequency of uterine torsion in the ewe and doe goat is very low, as it does not appear to be a significant cause of dystocia in any of the published surveys. However, this may reflect the limited veterinary involvement with ovine dystocia rather than a true difference in prevalence (Scott 2011). A lower prevalence in the sheep and doe compared with the cow may be related to differences of the anatomy of the broad ligament, whose attachment is sublumbar in the ewe rather than subileal as in cattle; it is therefore postulated that the pregnant uterus of the ewe would be less unstable than that of the cow.

In its clinical signs the condition closely resembles uterine torsion in cattle but, because of the smaller size of sheep and goats, it is much more difficult to insert a hand into the constricted vagina. Mild torsions may be confused with incomplete cervical dilation (Scott 2011). Diagnosis can be aided by the use of transabdominal ultrasonography (Wehrend et al. 2002, Jung & Bostedt 2007). The probe should be placed immediately cranial to the pelvic brim, with the probe head directed vertically (Scott 2012). The oedema of the uterine wall results in gross thickening of its ultrasonographic appearance.

There are various ways of treating the condition. The ewe or doe can be given caudal epidural anaesthesia (and maybe also clenbuterol) and, with the hindlimbs held so that the animal is almost vertical, a relatively modest rotatory force on a fetal appendage or a rotatory movement of the dam's body is usually sufficient to correct the torsion. Rolling, with a modification of Schäfer's method for the smaller body mass of the ewe, has been described as a means of managing uterine torsion (Kumar et al. 2016, Teja et al. 2017, Velladurai et al. 2017). If these methods fail then Caesarean surgery must be undertaken. Long-standing torsions can become impossible to resolve without resorting to surgery because of the development of adhesions between the uterus and other abdominal viscera.

Horse

Torsion of the uterus is an uncommon, but serious, condition of the pregnant mare. The prevalence is difficult to estimate, as most series are published from referral clinics where only the more difficult cases are seen. Ginther and Williams (1996) saw no cases of torsion among the 11% of dystocias recorded in 517 spontaneous foalings, whereas Leidl et al. (1993), in a study from the Munich Veterinary School, found uterine torsion present in 17 out of 100 dystocia cases. Chaney et al. (2006) reported 86 cases of uterine torsion between 1985 and 2005 in four referral hospitals and Vandeplassche et al. (1972) reported on 42 cases.

Uterine torsion is less common in the mare than the cow, perhaps due to the sublumbar attachment of the ovaries and dorsal attachment of the broad ligaments, which creates a more stable anatomical suspension of the equine reproductive tract than in the cow. The exact causes of uterine torsion are unknown, but suggested contributing factors include sudden falls or vigorous movement of a large fetus in a relatively small volume of fluid (Blanchard et al. 2010). Vandeplassche et al. (1972) found that twisting in an anticlockwise direction is the more common, and in the majority of cases, the extent of rotation was 360 degrees or more. However, of the cases reported by Riggs (2006), most were of 180 degrees.

Uterine torsion in the mare is much less strongly associated with parturition than it is in cattle: in the aforegoing surveys, more than half of the cases occurred before the end of gestation. Jung et al. (2007) reported uterine torsions that occurred between 5 months of gestation and term, whereas Pascoe et al. (1981) recorded the mean stage of gestation of mare with torsion as 9.6 months. Riggs (2006) considered that most cases occur in the last 60 days of gestation but before the onset of labour. The condition is therefore as much a differential diagnosis of colic as it is of dystocia: uterine torsions are commonly diagnosed as an acute event with the affected mare showing mild to moderate signs of colic (Riggs 2006). The possibility of uterine torsion should therefore always be considered in cases of colic during late pregnancy. Doyle et al. (2002) suggested that if any mare in late gestation shows signs of pyrexia, anaemia, tachycardia, and anorexia over a 2- to 4-week period, then chronic uterine torsion should be considered as one of the differential diagnoses. Diagnosis is readily established by rectal palpation of the crossed broad uterine ligaments, which also provides information on both the direction and degree of the torsion. The circulatory disturbance in the equine uterus is greater than with the same condition in cattle, with consequent risk to the survival of the fetus and the development of shock in the dam.

The main methods for correcting uterine torsion in the mare are: (i) vaginal correction in the standing mare; (ii) rolling the anaesthetised mare in lateral recumbency; (iii) flank laparotomy in the standing mare; and (iv) midline laparotomy in the anaesthetised mare.

If the mare is at full term and the cervix is sufficiently open to allow the fetus to be grasped, it may be possible to correct the torsion by rocking and applying rotational force to the fetus *per vaginam* (Riggs 2006). The ventrolateral aspect of the fetus is grasped, while the obstetrician's arm is resting on the dam's pelvic floor. The fetus is gently rocked until enough momentum is gathered to lift it up and over in the uterus in the direction opposite to that of the torsion. If successful, the fetus and uterus will rotate into the normal position and the mare will progress into second stage labour (Blanchard et al. 2010). Alternatively, the mare can be anaesthetised (e.g., xylazine/ketamine) and rolled in lateral recumbency by a method akin to Schäfer's method. The anaesthetised mare is placed into recumbency on the side of the torsion (right for clockwise, left for anticlockwise), a plank is placed over the dorsum of the abdomen to fix the calf, and the mare is then pulled over via dorsal recumbency onto her opposite side. This has proved to be a successful method of treatment: Wichtel et al. (1988) delivered six out of seven mares by this means, and

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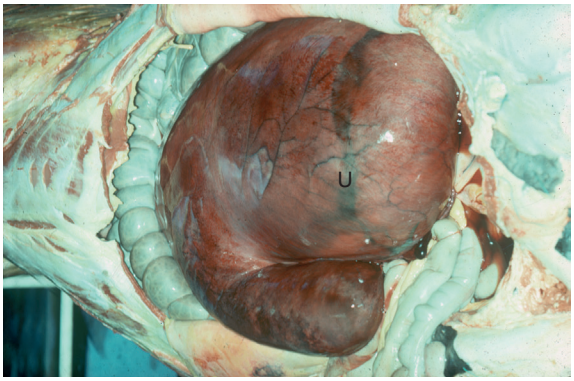

• **Fig. 13.8** Uterine torsion in a mare, as exposed by midline laparotomy. Note the congested uterus *(u)*. Correction by rotating the uterus was impossible, and a dead foal was removed after hysterotomy; thereafter, correction of the torsion was possible.

Dabas et al. (2014) corrected torsion in two mares that were 9½ months pregnant by rolling. These mares went on to deliver live foals at term.

Surgical correction should be used if rolling has failed to resolve the torsion after a couple of attempts or if the condition is deemed unsuitable for nonsurgical management. Flank laparotomy was recommended by Vandeplassche et al. (1972) to allow rotation of the uterus by direct traction. The mare is placed in the stocks, under just enough sedation to ensure that she remains standing. After preparation of the paralumbar fossa, local anaesthetic is infused in a line just cranial to the incision site or in a grid pattern (Vasey & Russel 2011). A high flank incision is made on the side of the torsion (Jones 1976, Threlfall 2007). The uterine torsion is corrected by reaching under the uterus, grasping the legs of the foal and gently rocking the foal and uterus with a pull-and-lift motion until it is returned to a normal position (Blanchard et al. 2010). If there is sufficient space, one hand can be used to grasp a fetal limb through the underside of the uterus and pull towards the operator, while the other hand simultaneously pushes against the dorsal aspect of the uterus. Occasionally, access through incisions in both flanks is required (Vasey & Russel 2011).

Alternatively, the mare can be anaesthetised and a midline laparotomy performed. Pascoe et al. (1981) suggested that recumbent laparotomy should be used if the mare is intractable, the uterus is ruptured, there is an emphysematous fetus, or the uterus is likely to be devitalised. In uncomplicated cases detorsion can be achieved by grasping the limbs of the foal and rotating the uterus. In complicated cases, such as when adhesions have formed (Doyle et al. 2002), the uterus is very friable or has ruptured, or the fetus is dead, removal of the fetus from the uterus is indicated before detorsion can be achieved (Fig. 13.8). However, Jung et al. (2007) left the (live) fetus *in utero* if fetal reflexes were present and provided intensive treatment of the mare to maintain the pregnancy. Seventeen of 23 mares managed that way carried to full term (two aborted, and four either failed to survive surgery or were euthanised). Saini et al. (2013) removed the fetus from the uterus of all 12 full-term/late-gestation mares with torsions, resulting in survival of 9 of the 12 mares, but only one foal survived long term.

Chaney et al. (2006) reviewed the factors affecting mare and foal survival in 63 cases of uterine torsion. Overall mare survival was 84% but was higher (97%) if the torsion occurred at <10 months of gestation than if torsion occurred later (65%). Overall foal survival (54%) was also higher if torsion occurred at <10 months gestation (72%) than later (32%). Foal survival was highest when the torsion was corrected by standing flank surgery, although this was confounded with gestational age. More advanced gestations or those selected for correction by midline laparotomy were already compromised by fetal hypoxia and/or devitalisation of the uterus.

When dystocia is due to uterine torsion, an attempt should be made to pass a hand through the cervix and to rotate the uterus by manipulating the fetus. According to Vandeplassche (1980), this is facilitated by the use of caudal epidural anaesthesia and raising the hindquarters of the mare. In addition, because of its recorded value in correcting uterine torsion *per vaginam* in the cow, clenbuterol might be used. If these methods fail, Caesarean surgery must be performed.

Pig

Uterine torsion is very rare in sows (Jackson 1973). However, when it does occur, it can be difficult to diagnose, and frequently, this is only done at postmortem examination. Torsion can involve one entire horn or, more frequently, part of one horn, thereby trapping a fetus or fetuses proximal to the obstruction; in time, the uterine wall will rupture and the fetus or fetuses will become pseudoectopic. It is one of the differential diagnoses to consider if a sow has not completed farrowing and yet piglets can either be palpated or identified using transabdominal ultrasonography. The only method of treatment is correction after a laparotomy, or probably a hysterectomy.

Dog and Cat

Uterine torsion is uncommon in the bitch. In a series of 182 dystocias examined at a veterinary hospital in Stockholm over a 4.5-year period, two cases of uterine torsion (1.1%) were diagnosed (Walett-Darvelid & Linde-Forsberg 1994). One was in a 1-year-old Mastiff, which had two macerated fetuses in which the complex torsion involved both horns and cervix, and the other case was in a 4-year-old Giant Schnauzer with 13 pups in which there was 180 degrees torsion. Both cases were treated by Caesarean surgery. Umamageswari et al. (2014) reported a unilateral torsion in a parturient German Shepherd bitch: two dead fetuses were located cranial to the torsion, and the uterine horn was necrotic. Likewise, Eyarefe et al. (2012) found 270 degrees torsion of one uterine horn, with four dead fetuses distal to the torsion. Torsion and haemometra of a bitch that had occurred in midgestation (33 days) was reported by Otava (2011). Murugavel et al. (2013) found uterine torsion in a bitch that also had pyometra. Subsequent pregnancies after treatment of unilateral or partial uterine torsion by partial hysterectomy have also been reported (Kumru et al. 2011). The clinical signs may be of an obstructive dystocia when pups remain in the uterus; however, it is very difficult to determine the precise cause of the obstruction. However, the bitch may also present with vague signs of malaise or, alternatively, of an acute abdominal disorder. In pregnant bitches a few instances have been discovered on postmortem examinations when there were torsions of up to 180 degrees; the rare finding of encapsulated fetal bones in a bitch's abdomen may be a legacy of uterine torsion and rupture. If a uterine torsion is promptly diagnosed, management by Caesarean surgery should be successful. The use of oxytocin may cause uterine rupture.

In the cat, torsions of 90 degrees to 180 degrees, involving either one horn or the uterine body, have been described in near full-term pregnancies or parturient queens (Freeman 1988, Ridyard et al.

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• **Fig. 13.9** Ventral hernia in a ewe. (Courtesy Dr. Rob Lofstedt.)

2000, Dal-Bo et al. 2013), typically in association with sudden illness. Midgestation torsion was described by Thilagar et al. (2005) in a queen that exhibited a bloody vaginal discharge and signs of an acute abdominal disorder, whereas postpartum torsion in association with puerperal metritis was reported by Oliveira et al. (2016). As in the bitch, a precise diagnosis of uterine torsion as a cause of an obstructive dystocia is difficult, even with good quality imaging techniques. Often a diagnosis is made only at laparotomy, in which case a prompt hysterotomy or hysterectomy should be performed. Occasional instances of extrauterine abdominal fetuses have been recorded (Bark et al. 1980). These probably result from uterine rupture during pregnancy, possibly associated with uterine torsion, rather than from an ectopic pregnancy.

Displacement of the Pregnant Uterus

Ventral Hernia in the Mare, Cow, and Ewe

Occasionally in all three species, hernia of the pregnant uterus occurs through a rupture of the prepubic tendon and muscles of abdominal wall (Fig. 13.9). The accident is one of advanced pregnancy, occurring at the ninth month or later in the mare, from the seventh month onwards in the cow, and during the last month in the ewe; it usually occurs in older or aged animals. The aetiology is obscure: a traumatic origin has been postulated, but it may also be attributed to abnormalities of posture (Seyrek-Intas et al. 2011) or intrinsic weakness of the musculature. Regardless of the underlying cause, the abdominal musculature becomes in some way so weakened that it is unable to support the gravid uterus. The site of the original rupture is the ventral aspect of the abdomen, a little to one side of the midline (left in the case of the mare and right in the cow and ewe) behind the umbilicus. It generally commences as a local swelling about the size of a football, but rapidly enlarges until it forms an enormous ventral swelling extending from the pelvic brim to the xiphisternum. It is most prominent at the caudal end of the abdomen, where it may sink to the level of the hocks. By this time, practically the whole of the uterus and its contents have passed out of the abdomen to occupy a subcutaneous position. In cattle the bulk of the swelling is often situated between the hind legs, the udder being deflected to one side. Generally, the condition is complicated by gross oedema of the abdominal wall due to pressure on the ventrally situated veins; in fact, this oedema may be so great that it is impossible to palpate either the edges of the rupture or the fetus.

As a rule, gestation is uninterrupted. Pregnant mares can be managed by supporting the caudal abdomen, stall rest, and/or analgesia. Schutten (2016) and Moaddab and Siavosh (2017) both described mares that successfully carried pregnancies to term and delivered the fetuses at full term. In the mare, delivery of the foal by traction, despite the downward deviation of the uterus, is possible; however, cases can be visualised in which displacement of the uterus places the fetus beyond reach. In these it is advised that the mare is anaesthetised and placed in dorsal recumbency, and the hernia reduced by pressure. Attempts at delivery should be made with the animal in this position. In the ewe, surgical reduction of ventral hernias has been described, along with delivery of the lambs by Caesarean surgery (Dar et al. 2016), and a similar procedure has been reported in the cow (Choudhury et al. 2016). Cows and ewes may give birth spontaneously despite severe ventral hernia, although affected animals should be closely watched during labour in case assistance is needed. Nevertheless, it is important to consider whether it is in the interest of the dam's welfare that the pregnancy should continue, or whether it might be preferable for the animal to be euthanised.

Downward Deviation of the Porcine Uterus

Downward deviation of the uterus has been described by Jackson (1973) as the cause of 19 of 200 cases of porcine dystocia. Affected animals strained vigorously despite an empty vagina, and at a point about 15 to 22 cm in front of the pelvic brim the uterus deviated sharply in a downward and backward direction. It was very difficult to extract the obstructed piglets manually, and insertion of the arm up to the shoulder was necessary so that the obstetrician's elbow could be flexed within the sow's abdomen. Affected sows were deep-bodied and pregnant with large litters.

Retroflexion of the Mare's Uterus

Vandeplassche (1980) reported 18 cases of retroflexion of the mare's uterus occurring over 10 years at the Ghent Veterinary Clinic, in which there was severe colic in mares near term with the foal occupying the maternal pelvis. *Per rectum,* the foal could be pushed back into the abdomen. However, this manipulation provoked renewed colic, and the fetus soon regained its intrapelvic position. It was found that the muscle relaxant isoxsuprine lactate in doses of 200 mg and injected at regular intervals relieved the colic and allowed the foal to move forwards in front of the pelvis. Normal parturition followed in due course.

Inguinal Hernia in the Bitch

In the bitch, acquired inguinal hernia, through which the uterus may herniate, is relatively common. The incarcerated uterus then often becomes the focus of pregnancy. The condition can also occur in the cat but is rare in this species. The hernia is generally unilateral and may contain one or both uterine horns.

Often the history is that an inguinal swelling the size of a hen's egg has been recognised by the owner for months, but that during the last few weeks it has rapidly become larger. In other cases the recent development of a progressive swelling is the story. There may or may not be a history of recent oestrus and mating.

The lesion is obvious and, if a careful examination is carried out, it is unlikely that it will be confused with a mammary neoplasm or a local abscess. The condition is painless, and there is no systemic disturbance. Although it is tense and irreducible, there is little tendency for strangulation, provided the intestine is not involved. The latter complication is rare. In those cases in which pregnancy is advanced, it will probably be possible to detect fetuses on palpation, which can be confirmed by transabdominal ultrasonography.

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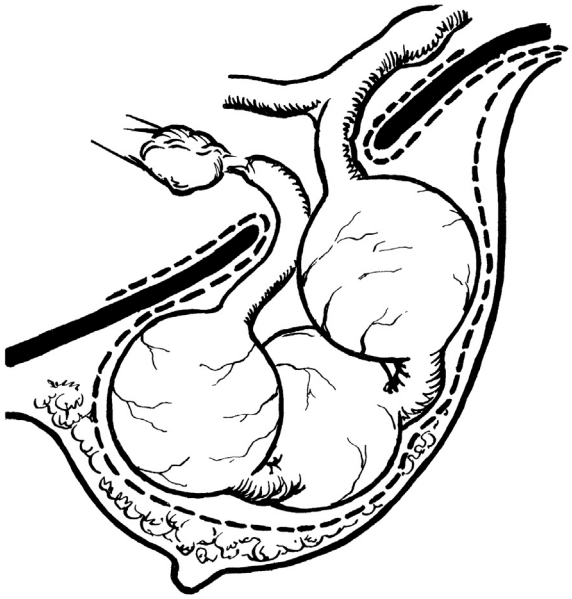

• **Fig. 13.10** Inguinal metrocele in a bitch gravid with three embryos of about 30 days.

The course of the condition depends primarily on the degree of tension in the hernial sac, and this will be influenced by its size and the number of fetuses involved. Sometimes, the fetuses will develop normally up to a certain point and then die, probably because of impaired blood supply to the herniated parts of the uterine horns, and then undergo resorption. The majority of cases will be presented when pregnancy has advanced about 30 days and each fetal unit is about the size of a golf ball, for by this time the size of the swelling is becoming alarming to the owner (Fig. 13.10). It is very unlikely, but not impossible, for such a pregnancy to go to term, with subsequent dystocia.

If an inguinal hernia is diagnosed in a pregnant bitch, these options should be considered:

- Reduce the hernia, obliterate the hernia sac, and allow pregnancy to take its normal course. In the vast majority of cases, it will not be possible to reduce the hernia by simple means.
- Enlarge the hernial ring by incision of the abdominal wall, obliterate the sac, suture the hernial ring closed, and allow the pregnancy to progress to term. This is the preferred method in terms of the outcomes of pregnancy and of the maintenance of the bitch's ability to breed. It presents, however, several technical difficulties. Precise incision of the abdominal wall forwards from the inguinal orifice is not easy owing to the presence of the large and tensely filled hernia sac. Moreover, effective closure of the neck of the sac may be difficult after incision of the parietal peritoneum.
- Dissect out the hernial sac, incise its apex, and expose the herniated uterus. Amputate the uterine horn involved. Obliterate the hernial sac. If it happens that the animal is also pregnant in an abdominally situated horn, this should be left intact. If, however, an abdominally situated horn is empty and it is desired that a hysterectomy be performed, it is relatively easy after locating the bifurcation to draw this horn into the hernia and remove it. As a rule, it is not possible to also draw the ovaries through the inguinal ring. This procedure is most often performed, as it presents no particular difficulties and cure of the hernia is certain.
- In those cases in which fetal development is at or approaching term, it may be decided to proceed as described previously but, instead of amputating the involved horn, perform a hysterotomy and extract the fetuses with their membranes.

Defects of Expulsive Forces

The expulsive force of labour is due to a combination of myometrial contractions and straining induced by the contraction of the abdominal muscles with a closed glottis. Because the abdominal muscles do not come into play until the myometrium has forced the fetus and fetal membranes into the pelvic canal and stimulated the pelvic sensory nerve receptors, it is logical to consider first the expulsive deficiencies that may arise in the myometrium. These may occur spontaneously or dependently and are called, respectively, primary and secondary uterine inertia.

Primary Uterine Inertia

The physiology of myometrial contractions during labour is described in Chapter 6. Primary uterine inertia implies an original deficiency in the contractile potential of the myometrium, thereby removing or reducing this component of the expulsive force and delaying or preventing the completion of the second stage of parturition. It is a common cause of dystocia in polytocous species, in which it has been shown to be responsible for 37% of dystocias in sows (Jackson 1973), 49% in bitches, and 37% in queen cats (Linde-Forsberg & Eneroth 1998). It also occurs relatively commonly in the cow, in which it is usually due to hypocalcaemia, as well as being a contributor to incomplete cervical dilation (see previous mention).

These factors may be involved in the cause of primary uterine inertia:

- The progesterone to oestrogen ratio is important as it influences uterine contractility in a number of ways (see also Chapter 6). Oestrogen increases: the synthesis of contractile protein; the number of agonist receptors for oxytocin and prostaglandins; the activity of myosin light-chain kinase (MLCK), which is involved in the phosphorylation of myosin and hence the biochemical changes involved in contraction; calmodulin synthesis, which increases MLCK activity; and the number of gap junctions. Progesterone has the opposite effects, thereby reducing myometrial contractility. In this context the findings of Bergström et al. (2010) are interesting, as they observed similar oestradiol concentrations in bitches with normal parturition and in those with uterine inertia, although the ratio of progesterone to prostaglandin $F_2\alpha$ (measured as PGFM) was higher in the animals with dystocia than in normal bitches. Such findings corroborate the notion of the dominant effect of progesterone in determining the onset and normality of labour. Others have suggested that partial failure of luteolysis may contribute to the development of uterine inertia in bitches.
- Oxytocin and prostaglandins are involved directly and indirectly in myometrial contractions. Any deficiencies in these hormones or their receptors, through which they exert their action, will prevent or reduce myometrial contractions.
- Calcium and related inorganic ions, such as magnesium, have a critical role in smooth muscle contractions. Any deficiency will impair these contractions, causing uterine inertia. This is a particular problem in dairy cows, particularly those at pasture because most cows experience a transient decline in food intake around the time of calving, which will result in reduced calcium intake. It is important to control feeding carefully during this

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transitional period because hypocalcaemia will cause uterine inertia, resulting in dystocia. Kraus and Schwab (1990) reported that bitches with primary inertia did not display calcium deficiency. However, Bergström et al. (2006) showed that, although low oxytocin concentrations were the direct cause of uterine inertia, the effects of low oxytocin were exacerbated by the presence of low concentrations of calcium. Discrepancies between results of the effects of calcium may reflect differences between total calcium and ionised calcium concentrations; Hollinshead et al. (2010) found that bitches, which were prone to uterine inertia, had higher concentrations of parathyroid hormone (PTH), lower concentrations of calcium, and higher blood pH than did normal bitches, suggesting that, by analogy with the similar situation in the cow, there may be merit in maintaining cationic/anionic balances in the diet of prepartum bitches.

- Overstretching of the myometrium, due to the presence of a large litter or excess fetal fluids (hydrallantois), or understretching, due to a small litter in polytocous species, can cause reduced uterine activity.
- There is anecdotal evidence that fatty infiltration between the layers of the myometrium can reduce its contractile efficiency.

The diagnosis of primary uterine inertia is made from the history and by an examination of the birth canal and presenting fetus. The dam is at or near term, as denoted by mammary changes and ligamentous relaxation in the pelvis (where this is normally apparent), although the psychological manifestations, coupled with restlessness due to abdominal discomfort, will have indicated that the first stage has passed. There may have been a few feeble abdominal contractions but no progress has been made or, in the polytocous species, after an adequate beginning of second stage labour all further activity has ceased. Linde-Forsberg and Eneroth (1998) refer to this as 'primary partial uterine inertia' to differentiate it from 'primary complete inertia' in which second stage fails to commence at all. It is difficult to distinguish this from secondary inertia, which is always a sequel to some other factor, such as an obstructive dystocia. Munnich and Kuchenmeister (2009) quantified this difference, finding 14% of dystocias were associated with a complete failure of myometrial contractions, compared with a total or 42% of dystocias in which 'inertia' of one form or another was diagnosed. Examination of the birth canal in the larger animals reveals a patent cervix, beyond which a fetus can normally be palpated, contained within its membranes. In the bitch and cat it is likely that no fetus or membranes will be felt.

It is essential that treatment should be attempted as soon as possible once other causes of dystocia have been ruled out as being responsible. In the large monotocous species, treatment is generally simple. By vaginal manipulation the membranes are ruptured and, if the fetus is in normal disposition, it should be delivered immediately by traction. In cows calcium borogluconate should be given even if there is no clinical evidence of hypocalcaemia.

In the sow there is evidence that hypocalcaemia is associated with some cases of uterine inertia (Framstad et al. 1989), but it is difficult to administer large volumes of calcium borogluconate as can be done in cattle. Treatment involves a combination of manual removal of any piglets that can be palpated in the vagina or uterus, together with the use of repeated doses of oxytocin. It is important to stress that oxytocin is a potent ecbolic, and doses of ≤10 IU i/m or ≤5 IU i/v should be used initially. Large doses tend to cause myometrial spasm rather than peristaltic contractions. In addition, the myometrium becomes refractory to repeated dosing, so it is important to provide an opportunity for an incremental dose regimen. The effect of different doses of oxytocin on the progress of parturition in sows has been described by Mota-Rojas et al. (2005, 2006) and Gonzalez-Lozano et al. (2009).

For the bitch and queen cat, in which primary uterine inertia is the main cause of dystocia, Linde-Forsberg and Eneroth (1998) suggested this treatment regimen:

- Vigorous exercise of the dam will sometimes stimulate uterine contractions.
- Digital stimulation of the vagina (feathering) will stimulate endogenous oxytocin release and may induce uterine contractions.
- Slowly inject 10% calcium borogluconate solution i/v (0.5–1.5 mL/kg body weight). This is in response to the notion that subclinical hypocalcaemia is a common cause of inertia.
- Leave the bitch for 30 minutes; if straining commences then repeat the calcium borogluconate treatment. If not, administer oxytocin at a dose rate of 0.5–5 IU i/v or 1 to 10 IU i/m in the bitch, depending on size, and 0.5 IU i/v or i/m in the queen.
- Perform a vaginal examination and remove any pups or kittens by gentle traction.
- Oxytocin treatment can be repeated, particularly if small numbers of the litter remain unborn.
- If calcium or oxytocin therapy is not successful or if the litter is very large or small (a single pup or kitten), then Caesarean surgery is indicated.

Nervous Voluntary Inhibition of Labour

In the series of canine dystocias reported by Freak (1962), in 17 of 272 cases, labour did not begin or, having begun, did not proceed. The factor common to all the affected bitches was the provision of a special parturition environment to which the bitch was unaccustomed. When the bitches were returned to their accustomed quarters, they proceeded to whelp. Birade et al. (2004), likewise, attributed uterine inertia to fear caused by firecrackers used in celebrations. Occasionally, bitches appear to be frightened by labour pains and voluntarily inhibit straining; tranquillising drugs are helpful in such cases.

Maternal excitement is a common condition of gilts, especially when they have been moved into a farrowing crate just before the onset of parturition. Gilts can become so excitable, aggressive, or disturbed that they appear unable to continue normal parturition. Sedation with azaperone (2 mg/kg i/m) leads to a resumption of normal farrowing. Oxytocin may also be given if there is any evidence of impaired uterine contractions. Azaperone is also used to manage maternal excitement that leads to litter savaging (Jackson & Cockcroft 2007).

Secondary Inertia

This is the inertia of exhaustion and is essentially a result, rather than a cause, of dystocia due to some other cause, usually of an obstructive nature. Nevertheless, in polytocous species, prolonged unsuccessful efforts to deliver one fetus may result in dystocia from inertia with regard to the remainder of the fetuses. Secondary inertia is frequently followed by retention of the fetal membranes and retarded involution of the uterus, factors that predispose to puerperal metritis.

Secondary inertia occurs in all species and, generally speaking, is a preventable condition. Its prevention depends on the early recognition that labour has ceased to be normal and the provision of appropriate assistance. Sometimes, in the bitch and queen

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cat, normal parturition will commence but, after expulsion of a few pups or kittens, it will then cease, even though there is no obstruction. Linde-Forsberg and Eneroth (1998) refer to this as 'primary partial inertia' and identify it as a major cause of dystocia, responsible for about 23% of the cases in both species in their study. It is very similar to the classical uterine inertia associated with large litters, making it difficult to distinguish between the two. If there has been an obstructive dystocia that has been corrected, and normal parturition has failed to resume, then this is clearly secondary uterine inertia.

In the monotocous species, correction of the cause of the dystocia that provoked the inertia is the essential feature of treatment. If this involves correction of faulty disposition, then the fetus should be removed immediately by traction. In the polytocous species, management of the case will depend on the duration of labour, the number of fetuses still unborn, and their condition. In an early case, delivery of the fetus causing the primary dystocia may be followed after a few hours by the return of uterine contractions, and parturition may proceed without further hindrance. Such is often the case in the sow and occasionally in the bitch and queen. When the case is of longer duration and there are still several young to be born, it is best to proceed with the delivery of the remainder. In the sow it is often possible to do this with the hand inserted into the uterus *per vaginam*. In the bitch it may be decided to attempt forceps delivery, but the protracted use of forceps when three or four fetuses remain unborn has very little to commend it. Calcium borogluconate and oxytocin therapy, as recommended for the treatment of primary uterine inertia, should also be tried. Because the fetuses will soon die, or may already be dead, an early decision on performing Caesarean surgery or hysterectomy is important.

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14

Fetal Dystocia in Livestock: Delivery *per vaginam*

TIMOTHY J. PARKINSON, JOS J. VERMUNT AND DAVID E. NOAKES

Manipulative Delivery *per vaginam*

Principles

The aim of manipulative procedures in the monotocous species and ewes/does with multiple fetuses must always be to ensure that the fetus is in normal disposition before attempting delivery. In polytocous species it is possible for *per vaginam* delivery to occur with some slight postural abnormality. Correction of defects of presentation, position, and posture can be achieved only by intrauterine manipulation of the fetus. Retropulsion of the fetus into the uterus is essential for repositioning it.

After diagnosing the cause of dystocia and deciding on a plan of action, the obstetrician should consider whether the available facilities are appropriate, whether there is sufficient professional and other help available, and whether the equipment is adequate to carry out the treatment successfully. In severe forms of dystocia, more especially in mares, the veterinarian should always seek the assistance of a professional colleague and consider whether it might be appropriate to transport the animal to a place with hospital facilities, provided that the animal is in a fit state to travel.

In cases of prolonged dystocia, fetal fluids and the lubrication of the birth canal become lost. Retropulsion into the uterus is greatly facilitated by substituting the lost fluids, and delivery is greatly facilitated by lubrication of the birth canal. Sterile water is the best alternative for allantoic fluid, although nonsterile clean water is perfectly satisfactory. In the cow and mare, volumes of up to 15 litres, instilled into the uterus by gravity using a soft rubber or plastic tube (a stomach tube is satisfactory) and funnel, will greatly increase the mobility of the fetus in the uterus. For actual vaginal delivery a lubricant substitute for the amniotic fluid is required, and this may be in the form of a water-soluble, cellulose-based obstetrical lubricant. If not available, a substitute such as soap (particularly soap flakes) is well tried and effective. The value of fetal fluid supplements cannot be too strongly emphasised.

Prevention of straining by the administration of caudal epidural anaesthesia greatly facilitates retropulsion of the fetus. In some circumstances there may be benefit to produce relaxation of the uterine muscle through the administration of a tocolytic drug such as clenbuterol.

Obstetrical manipulation *per vaginam* should be performed as cleanly as possible. It is impossible to perform them aseptically because there will inevitably be some contamination. It is important to sterilise or disinfect instruments and equipment between animals to minimise the contamination of the uterus and to prevent spread of diseases between animals.

Gentleness is of prime importance, so as to reduce the amount of trauma to the dam's genital tract and also to the newborn. Recent study has also focused upon the adverse effects of dystocia upon neonates, especially as the consequence of uncontrolled traction. The prevention of pain and discomfort is likewise of great importance. Caudal epidural anaesthesia, sedation, or general anaesthesia should always be considered as a means of managing pain. Some form of post-delivery analgesia should be administered as a routine after treatment of dystocia.

After the successful delivery of the fetus or fetuses, the dam's genital tract should always be examined for the presence of others, noting that monotocous species can have twins and, rarely, more. The dam's genital tract should be examined for signs of injury and appropriate treatment administered (see Chapter 20).

The fetus(es) should also be examined to see if resuscitation is necessary, if there is evidence of respiratory acidosis, which should be treated, and if there are injuries. A list of equipment for resuscitation of foals is described in Chapter 6.

Obstetrical Manoeuvres

Retropulsion

Retropulsion means pushing the fetus in a cranial direction from the vagina (and the bony pelvic canal) back into the uterus. It is fundamental to all intrauterine measures that may be required to rectify defects of presentation, position, and posture because there will be inadequate space within the pelvis to perform even the simplest of manipulations. It is effected by pressure with the hand on the presenting bulk of the fetus; in some cases it is convenient for an assistant to repel the fetus as the obstetrician otherwise manipulates it, whereas in others, retropulsion can be applied by means of a crutch (see Fig. 11.6G). As far as possible, the repelling force should be exerted in the intervals between bouts of abdominal straining. Alternatively, caudal epidural anaesthesia may be induced to prevent the dam straining; however, it has no effect on myometrial contractions, which can be suppressed by the use of a tocolytic such as clenbuterol.

Extension

Extension refers to the extension of flexed joints when postural defects are present. It is carried out by applying a tangential force to the end of the displaced extremity so that it is brought through

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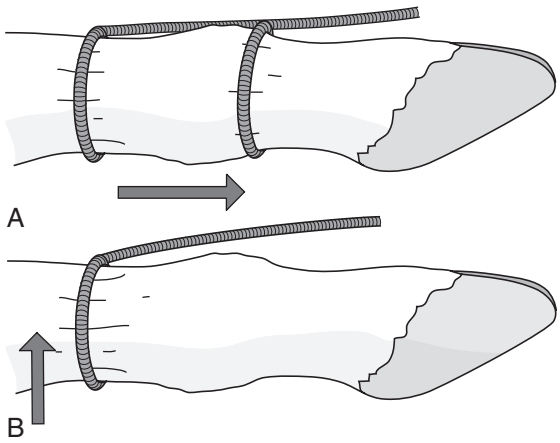


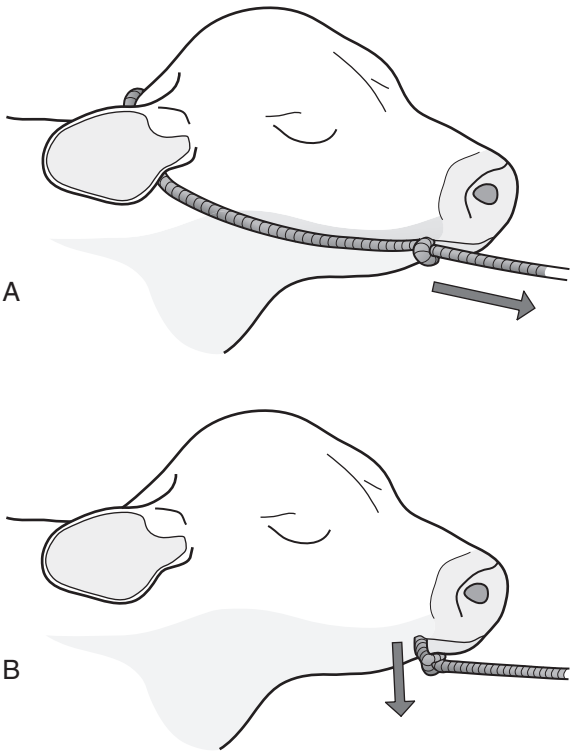
• **Fig. 14.1** (A) Correct placement of leg snares above and below the fetlock. (B) Incorrect placement only above the fetlock increases the risk of damage to that joint.

an arc of a circle to the entrance of the pelvis. The force should be applied preferably by hand or snares or, failing that, by hook(s).

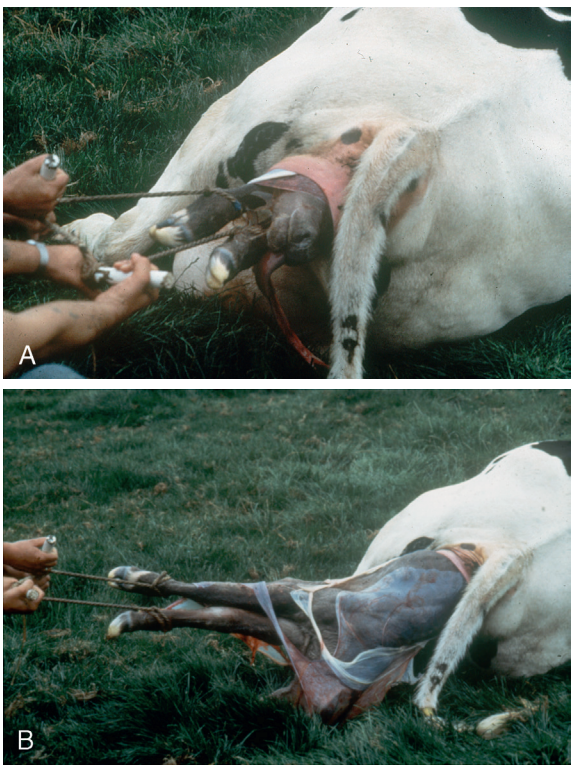
Traction

Traction means the application of force to the presenting parts of the fetus in order to supplement, or in some cases to replace, the maternal forces. Such force is applied by hand or through the medium of snares, hooks, or other mechanical traction devices. Limb snares should be double-hitched above and below the fetlocks (Fig. 14.1), and the head snare may be applied by the Benesch method, in which the loop is placed in the mouth and up over the poll and behind the ears (Fig. 14.2) or, alternatively, the centre of a single rope may be pushed up over the poll and behind both ears, leaving both ends of the rope protruding from the vagina. For replacement of a laterally deviated head, in which the operator's hand is insufficient, a thin rope snare applied to the mandible is valuable. However, this must only be used to correct the postural defect; traction, which might be used to effect delivery, must be applied by using a conventional Benesch head snare.

A very important consideration is the magnitude of the supplementary force that may be used because excessive force inappropriately applied can cause severe trauma to both dam and fetus. In the cow it is felt that the well-coordinated pull of four average persons should be the absolute limit. Fig. 14.3 shows traction being applied to a calf by two persons with rope snares; note the positioning of the loops of the snares proximal to the fetlock joint and the direction of the pull, which is along the normal path that the calf would take in a normal unassisted calving. Mechanical devices are now used extensively to apply traction; they must always be used carefully and sympathetically because they can cause severe trauma if used inappropriately. Table 14.1 gives some interesting data compiled by Hindson (1978), comparing the magnitude of the forces used to apply traction using a hydraulic drawbar dynamometer. This shows that calving jacks (or pullers) or pulley blocks generate over 5 or 6 times the force associated with a natural calving. However, for the stock person or veterinarian with little or no help, the pulley block and tackle or a calving aid, such as the HK calf puller or Vink calving jack (see Fig. 11.7), are invaluable. The most important aspect of applying effective traction is to coordinate the supplementary force with the straining effort of the



• **Fig. 14.2** (A) Correct placement of a head snare behind the ears and though the mouth (over the tongue). (B) Incorrect placement for applying traction. This position of a snare can, however, be useful for repositioning a laterally deviated head.



• **Fig. 14.3** (A and B) Traction by two persons, using rope snares to relieve dystocia in the cow.

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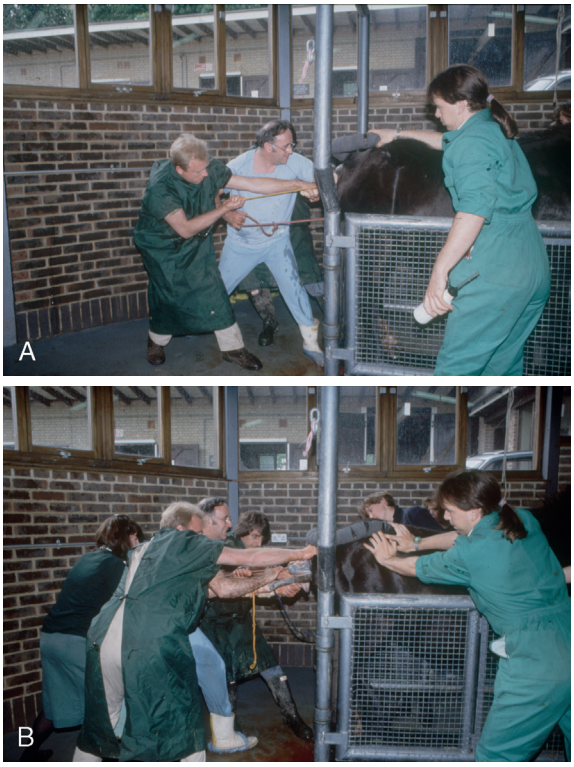
TABLE 14.1 Measurement of maximum tractive effort, as shown and recorded on a hydraulic drawbar dynamometer

Origin of Force	Tractive Effort (kg)
Cow at natural calving	70
Traction by one person	75
Traction by two persons	115
Traction by three persons	155
Calving jack	400
Pocket pulley blocks	445
Tractor	5000 +

Based on Hindson (1978).



• **Fig. 14.4** Traction by (A) two and (B) three persons to relieve dystocia in the mare.

dam. In the case of the cow, the slack in the calving snares should be 'taken up', as she strains, so preventing the calf from returning to its original site within the birth canal. Excessive traction is associated with fractured costochondral junctions of the ribs, tears of the diaphragm, and limb fractures in calves (Mee 1993) and foals (Schambourg et al. 2003).

In the mare the use of snares with two or three persons providing manual traction is usually sufficient (Fig. 14.4). In the ewe and doe goat, traction can be applied using simple fine cord snares or a fixed plastic head snare (see Fig. 11.9). In the sow, traction is nearly always applied using the hand, although fine cord snares and the plastic lambing snare can sometimes be very useful.

Rotation

Rotation entails alteration of the position of a fetus by moving it around its longitudinal axis, e.g., from a ventral to a dorsal position. It is more often required in horses than in cattle. To rotate the fetus, each forelimb should be secured with a snare and pulled into the birth canal. Apply copious lubrication. The most common method is to cross the limbs of the calf, one on top of the other, and try to rotate the calf. The head should be rotated at the same time. If a hand can be placed below the withers or below the shoulder joint of the fetus, so as to lift the chest upward and at the same time attempt to rotate the body, it may make rotation easier (Severidt et al. 2017). Mechanical force can be applied using of Cämmerer's torsion fork or Kühn's crutch. By repeating the process several times, it may be possible to rotate the fetus about 180 degrees.

Version

Version means the alteration of a transverse or vertical to a longitudinal presentation.

Sedation and Anaesthesia for Vaginal Delivery

Sedation, local analgesia, or general anaesthesia may be of benefit in managing obstetrical cases in terms of both ease of management of the case and the reduction of pain in the dam.

Sedation

Although most sedative drugs are not specifically licensed for use in pregnant mares, they can be particularly useful when dealing with a fractious animal. Although acepromazine has little or no effect on fetal cardiovascular function, it may not be very effective in sedating the mare. Detomidine and xylazine will effectively sedate the mare, but they affect fetal cardiovascular function and may reduce placental blood flow, with the obvious adverse consequences. If the fetus is already dead, xylazine may be the better of the two because it has a shorter duration of action (McGladdery 2001). Xylazine is the preferred sedative in cattle, either given by the IV route for a relatively short duration of action or by the IM route for a longer duration. However, the use of xylazine may cause contraction of the uterus, which may be unhelpful if further manipulations of the fetus are needed.

General Anaesthesia

General anaesthesia is better suited to the temperament of many mares than local analgesia, although in well-chosen cases epidural anaesthesia may be combined with sedation. When a complicated correction or fetotomy is required, it is best to use general anaesthesia, preferably in a veterinary hospital. Using hobbles and a hoist, it is relatively easy to place the mare in dorsal or lateral recumbency or to elevate her hindquarters. Such a change of position may greatly expedite obstetrical manoeuvres; in addition, elevation of the hindquarters will allow the foal to fall back into the uterus in the abdomen under the influence of gravity, thereby providing more space for any manipulative procedures. Because of pressure on the diaphragm, this technique may cause the anaesthetist some concern. Clarke et al. (2014) provide a thorough account of anaesthesia of obstetrical patients.

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Epidural Anaesthesia

Cattle

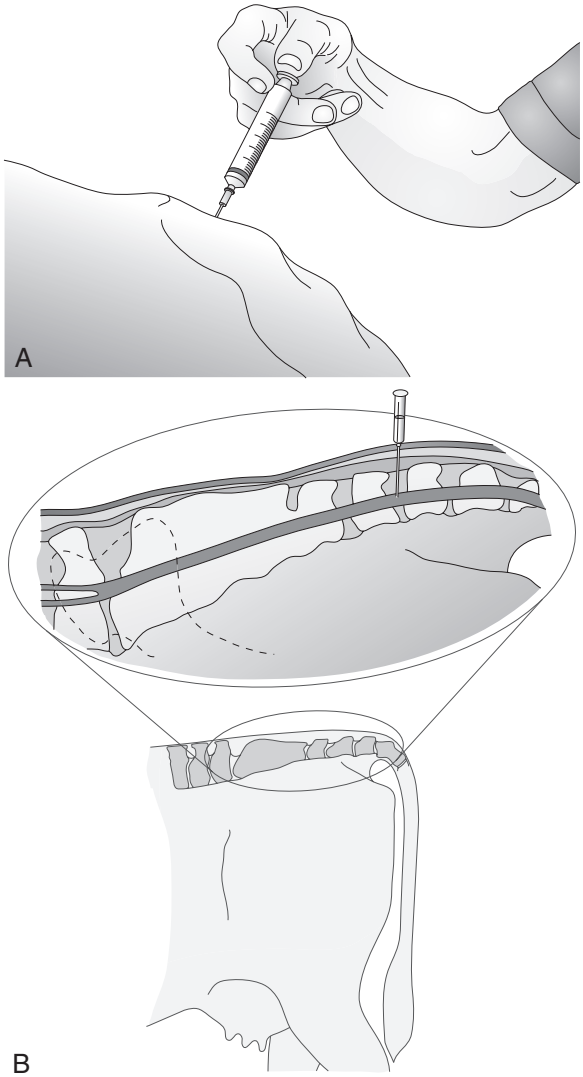
In cattle epidural anaesthesia is ideal for obstetrical purposes. It is a form of multiple spinal nerve block in which, by means of a single injection of local anaesthetic solution into the epidural space, the coccygeal and caudal sacral nerves are affected, thus producing anaesthesia of the anus, perineum, vulva, and vagina. The key advantage to the veterinarian is that, by abolishing pelvic sensation, reflex abdominal contraction (straining) is prevented. Thus intravaginal manipulations are facilitated, retropulsion is made easier, fetal fluid supplements are better retained, and faecal contamination of the perineum is reduced. Epidural anaesthesia also reduces pain in the dam, so the patient stands more quietly and will rise more quickly from recumbency once the delivery is completed. This form of anaesthesia is useful whenever straining is troublesome, as in prolapse of the uterus, vagina, rectum, or bladder. It is also indicated for episiotomy and for suturing lacerations of the vulva or perineum.

Provided that the epidural injection is made with due regard to asepsis and that an excessive volume of anaesthetic is not injected (thus causing the animal to become recumbent), the method is free from risk. It should be clearly understood that epidural anaesthesia does not inhibit myometrial contractions; it has no effect on the third stage of labour or uterine involution.

The preferred site of injection is the middle of the first intercoccygeal space (Fig. 14.5). This is located by raising and lowering ('pumping') the tail to identify the first obvious articulation behind the sacrum. The sacrococcygeal space can also be used; however, it is smaller than the first coccygeal space and in some older cows becomes ossified. The spinal cord and meninges are cranial to these points; the spinal canal contains only the coccygeal nerves, the thin phylum terminale, vasculature, epidural fat and connective tissue.

The area is clipped, thoroughly washed with an antiseptic solution or surgical scrub and dressed with surgical spirit. Some inject a small volume of local anaesthetic, using a fine needle, to desensitise the skin over the injection site; others do not. The epidural needle, which is 18 to 20 gauge and 2.5 to 5 cm long, is inserted into the middle of the space at right angles to the normal contour of the tailhead, exactly in the midline and directed downwards in the midsagittal plane. It is easier to ensure that this occurs by standing directly behind the cow. Some find it easier to direct the needle slightly cranially at an angle of about 10 degrees to 30 degrees from the vertical. The needle is passed downwards for a distance of 2 to 4 cm until it strikes the floor of the epidural space; it is then very slightly withdrawn. Confirmation that the needle is correctly placed is obtained by attaching to it the syringe and making a trial injection; if there is no resistance to injection, the needle point is in the epidural space. Alternatively, the hub of the epidural needle can be filled with anaesthetic solution. As the needle is advanced into the epidural space, the anaesthetic solution will be sucked in as a result of the slight negative pressure that exists there (the so-called 'hanging drop' technique). The tail becomes limp almost immediately, but it takes a slightly longer time interval (up to 10 min) before the perineum is desensitised and the straining reflex is completely abolished.

A dose rate of 1.0 mL/100 kg of 2% lignocaine hydrochloride injected at a rate of 1 mL per second will produce obstetrical anaesthesia lasting about 30 to 60 minutes; thus heifers and small cows require a volume of 5 mL and large cows 7 to 8 mL (Skarda 1996). Fat deposition in the epidural space of cows in high body condition reduces the effective volume of the space, so the amount of local anaesthetic should be reduced in such animals. Generally,



• **Fig. 14.5** Epidural anaesthesia in the cow. (A) Site of injection between vertebrae C1 and C2. (B) Longitudinal section through the caudal vertebrae of the cow, showing the relationship of the coccygeal vertebrae to the pelvis.

it is preferable to administer approximately 5 mL, then to 'top up' to effect. Giving too much local anaesthetic will, of course, affect the motor nerves of the lumbosacral plexus, resulting in long-term recumbency of the cow. The simultaneous injection of xylazine into the epidural space at a dose rate of 0.05 mg/kg diluted with 2% lignocaine to a volume of 5 mL can prolong the duration of anaesthesia for up to 3 hours (Grubb et al. 2002). Mild to moderate sedation typically also occurs as a result of epidural administration of xylazine, which can be controlled by the intravenous injection of tolazoline hydrochloride, an α_2-adrenoceptor antagonist, at a dose rate of 0.3 mg/kg (Skarda et al. 1990).

Sheep and Goat

Caudal epidural anaesthesia is a very useful, if somewhat underutilised, technique in ovine and caprine obstetrics because, although in both species there will be straining, the relative forces are much less than in the cow, and the ewe or doe can always be suspended by her hindlimbs. However, the uterus of both species appears to be more susceptible to rupture when manipulative procedures are performed. For this reason, and in the interests of welfare,

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anaesthesia should be used as a routine for all but the simplest of vaginal and uterine manipulations.

The injection can be made into either the sacrococcygeal or the first coccygeal interspace with a 20 gauge × 2.5 cm needle, using 2% lignocaine hydrochloride at a dose rate of 0.5 mg/kg (~2 mL for an 80 kg ewe) to provide caudal analgesia for up to 4 hours. The incorporation of 0.07 mg/kg xylazine (~0.25 mL of 2% xylaxine for an adult ewe) will prolong analgesia for up to 36 hours (Scott 1996). Ismail (2016) has recently reviewed other drugs that can be combined with various local anaesthetics to improve the quality or duration of analgesia.

Horse

The technique of epidural injection in the mare has been described by Robinson and Natalini (2002). The technique is similar to that in the cow, but, because the root of the tail is well covered by muscle and fat, the spines of all coccygeal vertebrae are more difficult to locate. The first coccygeal interspace is the preferred site. An imaginary line joining the two hip joints crosses the midline of the sacrococcygeal joint. The spinous process of the first coccygeal bone and, caudally, the first coccygeal interspace can be palpated in thinner horses. The first coccygeal interspace corresponds often with the first moveable joint in the tail and can be recognised when the tail is raised and lowered. It lies approximately 2.5 to 5 cm cranial to the origin of the tail hairs. It can also be recognised as being at the level of the caudal skin folds that are situated on each side of the tail (Robinson & Natalini 2002). It is important to ensure that the mare is adequately restrained. After the site has been clipped and thoroughly cleaned, a small bleb of local anaesthetic should be injected subcutaneously and into the surrounding tissue over the site. With the mare standing squarely and symmetrically, an 18 gauge × 4 to 8 cm needle (note that the needle needs to be longer than in the cow) should be inserted at about 10 degrees from the vertical and directed cranially until it strikes the floor of the spinal canal; it should then be withdrawn 0.5 cm before the injection is made (Fig. 14.6).

Traditionally, 2% lignocaine hydrochloride is effective, using a volume of 6 to 8 mL in a light hunter-type mare weighing 450 kg; proportionately larger or smaller volumes should be used in larger and smaller animals, respectively. It is important to err on the side of caution because too large a volume will cause ataxia, which is of much greater concern in horses than in cattle because of their different temperaments. An additional volume can be given if the needle is capped and left in place. It also takes longer for the anaesthesia to take effect in the horse than the cow. Other local anaesthetic agents can be used (in different volumes), as well as α_2–adrenoceptor agonists such as xylazine (0.17 mg/kg) and detomidine (60 µg/kg) in 10 mL 0.9% saline; the latter is used either alone or in combination with a local anaesthetic. A rapid-onset (5 minutes) and long-lasting (5 hours) caudal epidural anaesthesia was obtained, using a combination of 2% lignocaine hydrochloride (0.22 mg/kg) and 2% xylazine (0.17 mg/kg) (Skarda 1996).

Pig

Epidural anaesthesia is rarely used for obstetrical purposes in pigs other than for replacing prolapses of the vagina and uterus. The site of injection is the lumbosacral space, which can be located as follows: the wings of the ilia are joined by an imaginary transverse line; where this crosses the middorsal line, the needle is inserted perpendicularly and then directed forward at an angle of 20 degrees from the vertical until it strikes the floor of the vertebral canal. The needle is then withdrawn slightly and the injection made. The size of the needle varies depending on the size of the gilt or sow, but over 100 kg body weight, an 18 gauge 10 to 15 cm needle is satisfactory. The sow or gilt needs to be adequately restrained, preferably in a crate to prevent lateral movement (which can be the most difficult aspect of the technique and can make the difference between success and failure). For anaesthesia caudal to the umbilicus, a dose rate of 1.0 mL of 2% lignocaine hydrochloride per 4.5 kg body weight injected at 1.0 mL per 2 to 3 seconds should achieve anaesthesia by 10 minutes and last for about 120 minutes (Skarda 1996). It should be noted that injection at this site affects the nerves of the lumbosacral plexus and will cause posterior paralysis.

• **Fig. 14.6** Site of epidural anaesthesia in the mare.

Dystocia Due to Postural Defects: Anterior Presentation

Faulty disposition due to postural defects are a frequent cause of dystocia. Postural defects can usually be corrected by manipulation if they are treated early in second stage labour. However, in neglected cases associated with secondary uterine inertia, loss of fetal fluids, and a dead, emphysematous fetus that is tightly enclosed by the uterus, very serious dystocia may occur. Manipulative delivery for such cases is often not possible and can be difficult to resolve, even by fetotomy or Caesarean hysterotomy.

The mechanics of the correction of postural defects are simple in principle (although not always so simple in their application). Correction of all postural defects requires that the fetus is repelled into the uterus, as there is insufficient space within the pelvis/birth canal to reposition limbs. In turn, except for dystocias of short duration, retropulsion will require epidural anaesthesia, substitution

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of lost fetal fluids, and/or lubrication of the birth canal. If the uterus is tightly contracted over the fetus, administration of a tocolytic drug, such as clenbuterol, may also be indicated. After epidural anaesthesia and correction of the posture, the fetus must then be delivered by traction because the cow will not strain to aid expulsion. If it is possible to use both arms simultaneously inside the cow (i.e., one to push and the other to pull), it may be easier to correct a postural defect.

Postural Defects of Anterior Presentation in Cattle

Carpal Flexion Posture

One or both forelimbs may be affected. In the unilateral case the flexed carpus is engaged at the pelvic inlet; the other forefoot may be visible at the vulva. The simple recent case requires retropulsion at the fetal head or shoulder; the retained foot is then grasped (by cupping the digits in the hand) and, as the carpus is pushed/lifted upwards, the foot is carried outwards and finally brought forwards in an arc over the pelvic brim and extended alongside the other limb (Fig. 14.7A). More difficult cases require a snare attached to the retained fetlock to help extend the limb (see Fig. 14.7B). The fetal foot should always be carried over the pelvic brim in the cupped hand of the obstetrician (see Fig. 14.7C). More neglected cases may require substitution of fetal fluids and the use of lubricant to help mobilise the calf. Rarely, in very protracted dystocia or in cases of ankylosis, the limb cannot be extended in which case it must be cut through above the carpus by means of the wire saw fetotome (see Chapter 15).

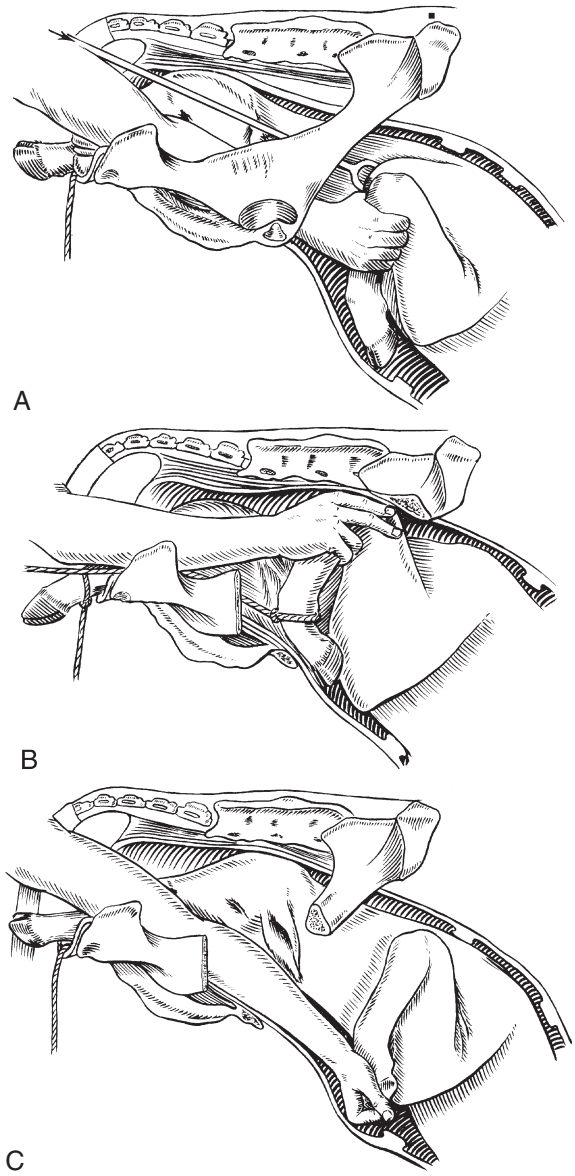
Incomplete Extension of the Elbow

This case is diagnosed on vaginal examination, with the digits emerging at the same level as the fetal muzzle instead of being well advanced beyond it (Fig. 14.8). Usually, the calf can be repelled by pushing on the head, after which each limb is pulled in turn in an obliquely upward direction, so as to lift the olecranon process over the maternal pelvic brim. Delivery is accomplished by traction on the head and both forelimbs.

Shoulder Flexion Posture: Complete Retention of the Forelimb(s)

This type of dystocia may be unilateral or bilateral. The diagnosis of bilateral retention is usually obvious by observing that the head partly or completely protrudes from the vulva, but in the absence of the forelimbs. In case of bilateral carpal flexion, the head cannot be advanced that far (Fig. 14.9A).

In a 'roomy' cow, with a small full-term or premature calf, the dystocia may be overcome by traction in the abnormal posture; in such cases, unless there has been much delay, correction of the abnormal posture is relatively easy. More usually, the malposture has to be corrected, which requires retropulsion of the fetus. It is, however, common for the protruded head to be very swollen and impossible to repel. When this occurs, there is little alternative but to amputate the head outside the vulva. To achieve this, eye hooks or the points of the Krey's hook are placed in the orbits (see Fig. 14.9B), and traction or downward pressure is applied to the head, so that the head can be forced completely beyond the vulva. It is then amputated either by disarticulation at the occipito-atlantal joint, using a sharp knife or scalpel, or by cutting through the neck (as close as possible to the calf's trunk), using a wire saw fetotome. As the fetus is then repelled, the retained forelimbs tend to come forwards; the calf's radius and ulna are then grasped and the defect is converted into carpal flexion posture and relieved accordingly (Fig. 14.10A and B).



• **Fig. 14.7** (A) Disposition of the calf: anterior presentation, dorsal position, unilateral carpal flexion posture. Correction using a hand and a crutch. (B) Correction using a hand and a digital snare. (C) Correction by hand alone. Note the method of grasping the foot.

In the more difficult case, the limb must be snared, at first proximally and then the noose passed down until it lies above the fetlock, the shank being placed from before backwards between the digits so as to flex the fetlock and pastern when traction is applied to it (Fig. 14.11). The digits are held in the cupped hand and the carpus is forced upwards while an assistant, pulling on the snare, helps the operator to bring the foot over the pelvic brim. In a delayed case, such a manoeuvre may be impossible and then removal of the limb is undertaken by percutaneous fetotomy (see Chapter 15).

Lateral Deviation of the Head

Lateral deviation of the head is a very common form of ruminant dystocia. The head may be displaced to either side. The presentation

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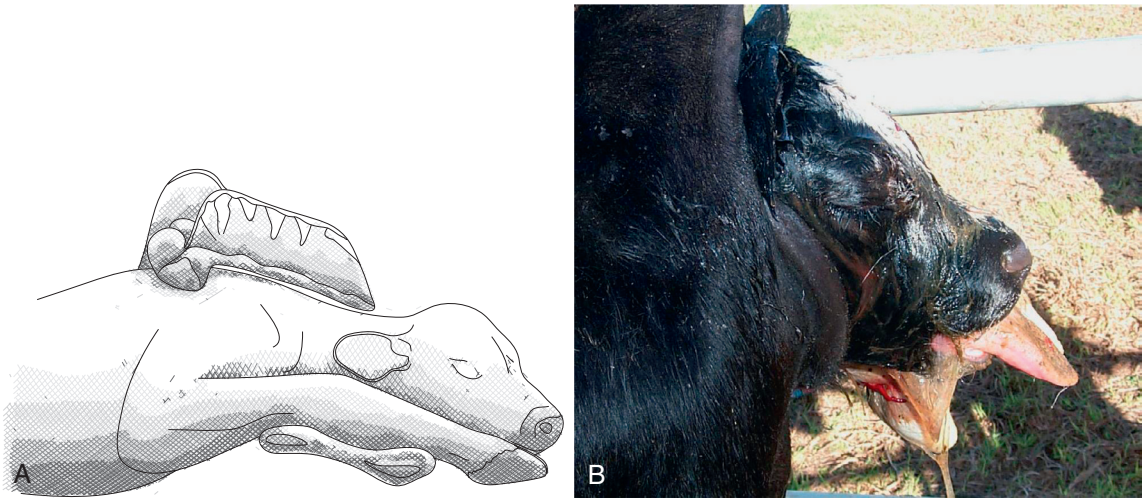
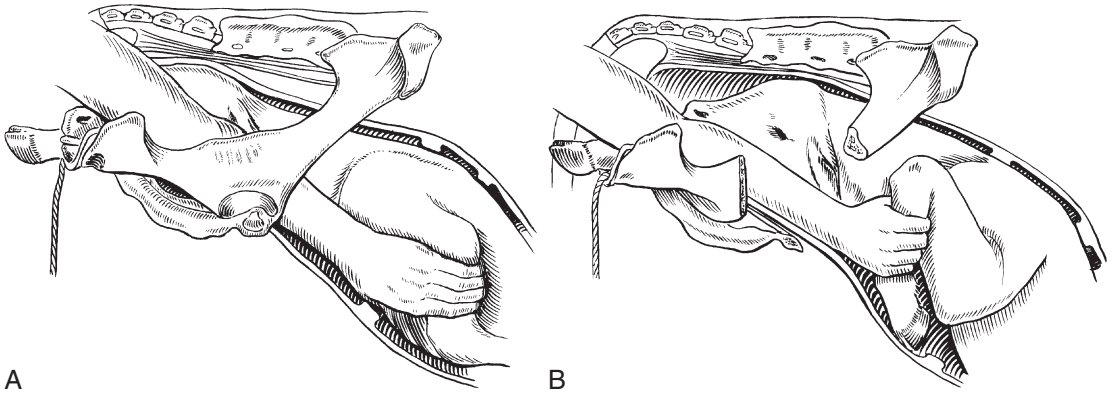


• **Fig. 14.8** (A) Disposition of the calf: anterior presentation, dorsal position, incomplete extension of elbow posture. (B) Presentation of a calf with incomplete extension of elbows. (Courtesy Dr. Jakob Malmo.)

• **Fig. 14.9** (A) Presentation of a calf with unilateral flexion of the shoulder. The head and one limb are protruding from the vulva. (B) Placement of eye hooks in the orbits before removal of the head of a dead calf with bilateral shoulder flexion. (Courtesy Dr. Jakob Malmo.)



• **Fig. 14.10** Disposition of the calf: anterior presentation, dorsal position, unilateral shoulder flexion posture (complete retention of the forelimb). (A) First stage of correction by hand. (B) Second stage of correction by hand.

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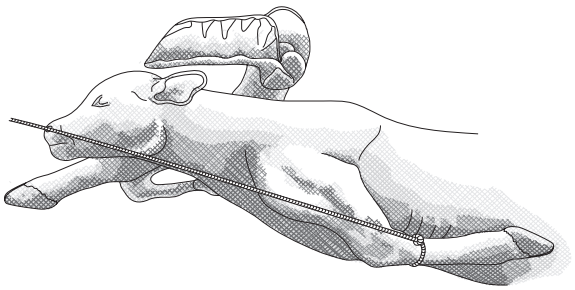


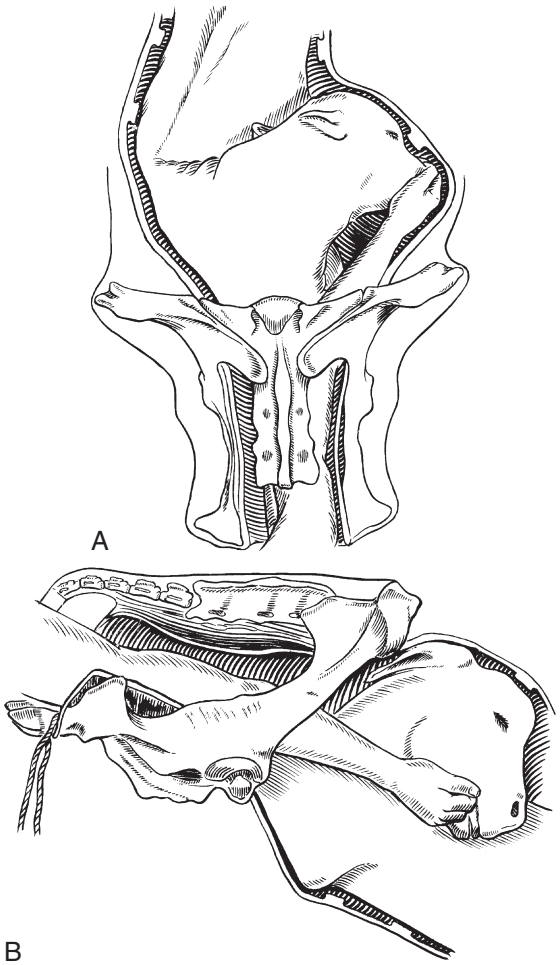
• **Fig. 14.11** Disposition of the calf: anterior presentation, dorsal position, unilateral shoulder flexion posture. Correction using a snare that has been pushed down to below the carpus.

is typically of both forelimbs presented at the vulva, but, on vaginal examination, the head is not present in the birth canal. Of course, one should ensure that the calf is not misdiagnosed as being in posterior presentation. When presented in early second stage labour, this malposture can often be corrected by hand. The lubricated hand is introduced and the fetus is repelled by pushing forwards at the base of its neck. The hand is then quickly transferred to the muzzle of the calf, which is firmly grasped and brought round through an arc until the nose is in line with the birth canal (Fig. 14.12A). During this action it is important that the muzzle is fully cupped in the hand to protect the uterine wall from the sharp incisor teeth of the fetus. In a more inaccessible case, the muzzle may be reached after preliminary traction on the commissure of the mouth (see Fig. 14.12B) or on the mandible. It is important to place a head snare as well as forelimb snares before the fetus is delivered by traction.

In more protracted cases of dystocia due to head displacement, with greater loss of fetal fluid and with the uterus contracted on the calf, it is more difficult to rectify the posture. Replacement of fetal fluids, lubrication, and epidural anaesthesia are all required. A special head cord of smaller calibre than those used on the limbs is carried in as a running noose and slipped over the mandible of the calf, where it is tightened, and the shank of the snare is handed to an assistant (Fig. 14.13A). The operator reintroduces a hand, grasps the calf's muzzle, and, as it is manipulated to extend the neck, the assistant is directed to apply gentle traction. It is obviously important that this head snare should be passed around the greater curvature of the neck to the mandible. Should the snare inadvertently be passed across the concavity of the neck curvature to the mandible, pulling on it will accentuate, rather than relieve, the displacement (see Fig. 14.13B and C). Whichever method is used to correct this malposture, it is important to protect the tubular genital tract as the neck is extended from the possibility of cuts by the sharp lower incisor teeth of the calf; this is best done by cupping the hand over the calf's mouth. The correction of this postural defect can also be facilitated by casting the cow in lateral recumbency on the side opposite to the direction of the neck flexion; this allows the gravid uterus to sink slightly to one side, thereby providing more space to correct the deviation.

Ventral Deviation of the Head

This is an uncommon type of dystocia in cattle. It usually takes the form of 'vertex posture' in which the calf's nose abuts on the pubic brim and the brow is directed into the pelvis (Fig. 14.14A). The more severe varieties of downward deviation of the head, namely 'nape presentation' and 'breast–head' posture (in which the head is flexed vertically between the forelimbs (see Fig. 14.14B)) are rare



• **Fig. 14.12** Disposition of calf: anterior presentation, dorsal position, lateral deviation of the head. (A) Correction by hand. (B) Preliminary 'hooking' of the commissure of the mouth before grasping the muzzle of the calf.

in cattle; when present, they have usually been caused by traction on the limbs before the head was fully extended.

Provided sufficient retropulsion can be achieved, vertex posture is easily overcome. Neglected cases are likely to require caudal epidural anaesthesia and fetal fluid replacement. The calf is repelled by applying pressure to the forehead with one hand (or the thumb) while lifting the mandible over the pelvic brim with the other hand (or the fingers of the same hand).

More severe degrees of downward displacement of the head are treated in a similar way, but, if difficulty is experienced, returning one or both of the forelimbs back into the uterus may provide enough room to rotate the head. In difficult cases of downward deviation in which the calf is still alive, Caesarean hysterotomy should be considered. If the calf is dead, then the head and neck should be removed by means of percutaneous fetotomy.

Postural Defects of Anterior Presentation in Horses

Although showing a lower incidence in horses than in cattle, defects of limb posture cause more serious dystocia in mares than in cows. This is due to the severe pelvic impaction that is consequent upon the mare's very strong expulsive efforts and to the longer limbs of

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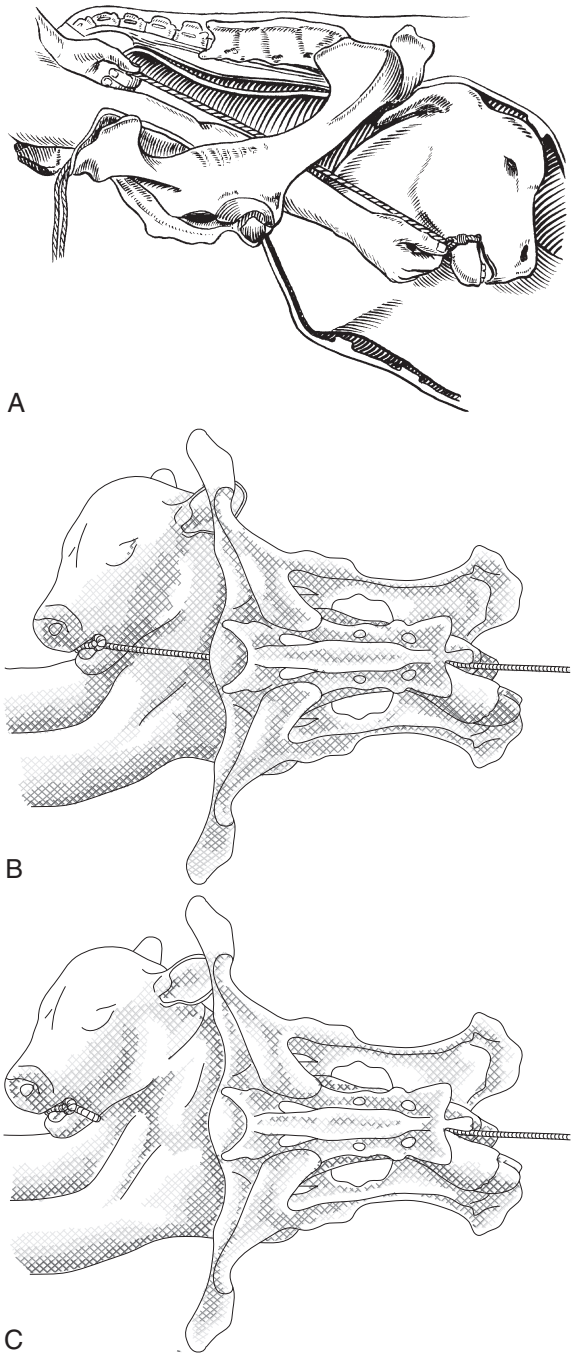
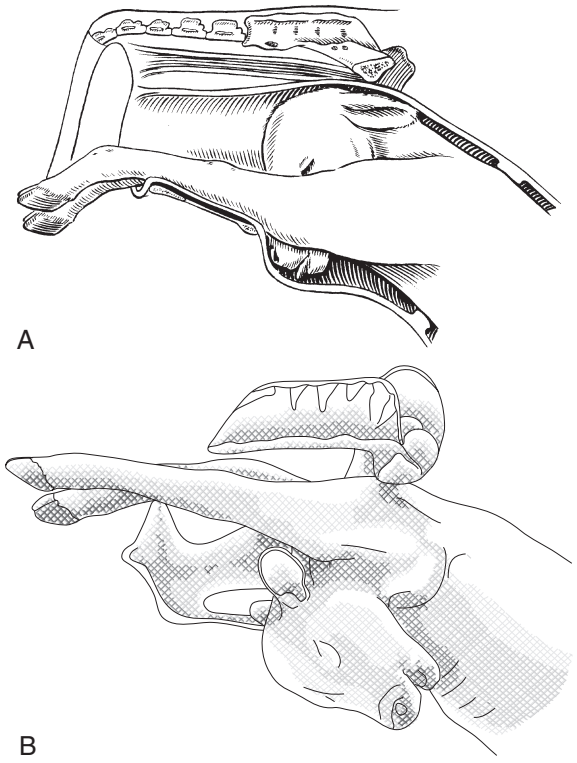


• **Fig. 14.13** Disposition of calf: anterior presentation, dorsal position, lateral deviation of the head. (A) Application of the mandibular snare. (B) Incorrect direction of traction, which will exacerbate rather than relieve the dystocia. (C) Correct direction of traction, with the snare passed around the greater curvature of the neck.



• **Fig. 14.14** (A) Disposition of the calf: anterior presentation, dorsal posture, downward displacement of the head ('vertex posture'). (B) The 'breast head' posture.

the foal. The principles of correction are the same as for the cow, but adequate retropulsion of the fetus is essential in order to create sufficient room for extending the longer limbs of the foal. Foot snares are great aids to manual extension of the limb. In order to prevent rupture of the uterus or vagina, correction of posture must be done with the utmost care. Although the obstetrician is ever mindful of the urgency of equine dystocia, if an impacted fetus is found to be already dead, the advantage of anaesthetising the mare and placing her in lateral or dorsal recumbency should be considered.

Carpal Flexion Posture

If only one limb is affected, the fetal nose and one forelimb are present at the vulva, but the mare makes no progress with delivery despite straining. The retained limb may be present at the pelvic inlet, or the flexed carpus may be pushed up into the vagina so that it comes almost up to the level of the vulva.

The principles of correction are the same as for the cow: the fetus is repelled as far as possible, and the carpus is pushed upwards and forwards to bring the foot within reach. The carpus is rotated dorsolaterally, and the flexed fetlock is brought medially and caudally into the birth canal (Frazer 2011). During the final extension of the limb the birth canal is protected from injury by holding the fetal foot in the cupped hand. There is a tendency for a foal in carpal flexion posture to become impacted in the maternal pelvis (Fig. 14.15A); the procedure required will depend on the degree of impaction, on the relative size of both the fetus and the birth canal, and on the duration of second stage labour. For irreducible carpal flexion in a dead foal fetotomy is undertaken by means of the wire saw fetotome, the section being made through the carpal joint (see Chapter 15). A snare is then placed above the carpus and the fetus is delivered by pulling on this as well as on the other limb and head. Bilateral carpal flexion can be managed by unflexing each limb in turn. However in the case of an irreducible bilateral carpal flexion affecting a normally developed full-term foal, it is unlikely that the foal will still be alive so that fetotomy is indicated, one or both carpal joints being sectioned as required. Pathological contracture of the tendons can also hinder unflexing of the carpus: one or both joints may be affected (Vandeplassche 1987). Some

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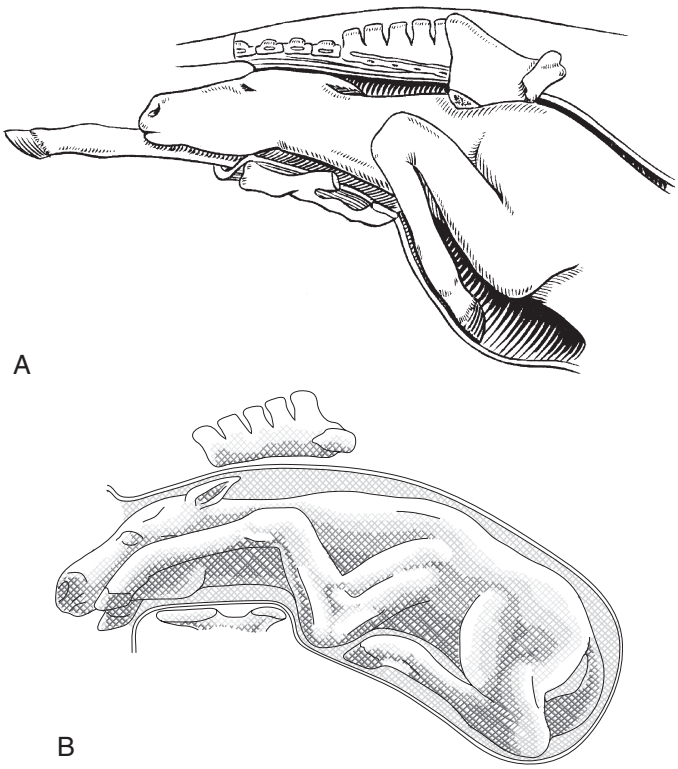


• **Fig. 14.15** (A) Disposition of the foal: anterior presentation, dorsal position, unilateral carpal flexion posture. Note the tendency to impaction in this malposture. (B) Disposition of the foal: anterior presentation, dorsal position, incomplete extension of elbow posture. Note the impaction of the elbows below the pubis.

of these cases may be resolved by repositioning, but more severe cases will require removal of the flexed limb by fetotomy.

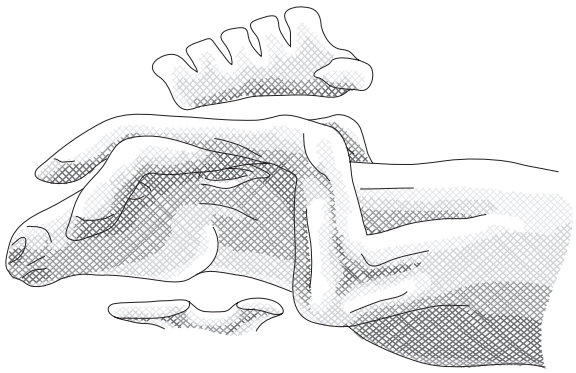
Incomplete Extension of the Elbow

This condition is uncommon. As in the cow, the fetal nose and tips of the feet are presented together at the vulva such that the feet are not extended beyond the nose as would be the case in a normal delivery (Figure 14.15B). The condition is relatively easy to correct, by repelling the fetus and pulling each limb in turn into a fully extended position.

Shoulder Flexion Posture

It is much more common for one than both shoulders to be flexed. When one forelimb is retained, the head and one forelimb are presented at the vulva. The second forelimb is absent, but the shoulder joint and proximal parts of the limb can be palpated on that side. When both shoulders are flexed, only the head is presented at the vulva. The more slender head and longer neck of the foal allows for more room in the maternal pelvis for the hand and arm of the obstetrician than is available in the same type of bovine dystocia, but the great length of the forelimbs makes manual repositioning of the foreleg difficult. Copious amounts of fetal fluid supplement should be infused and the fetus repelled. The limb is grasped around the humerus, and the hand, or a snare, is advanced to the radius. Extension of the shoulder is difficult (Steiner et al. 2008), requiring that it is moved medially and caudally and then elevated in order to achieve a carpal flexion posture. If a snare can be placed around the radius, one hand can be used to apply traction to the distal limb while the other repels the fetus (Frazer 2011). Once converted into a carpal flexion, it can be corrected into normal posture. Anaesthesia of the mare and elevation of her hindquarters may aid in correcting the malposture.

The difficulty of correcting shoulder flexion means that, when both legs are involved and the fetus is alive, Caesarean hysterotomy is indicated. Likewise, for many cases of unilateral flexion, Caesarean hysterotomy may be preferable to lengthy manipulation. In some mares with a large pelvis and which are carrying a relatively small foal, it may be possible to extract the fetus with one shoulder flexed. However, this method poses significant risk of trauma to the birth canal of the mare and of rib fractures in the foal. If the fetus is dead and it is impossible to retrieve the retained foreleg, it can be removed by fetotomy. The fetotomy wire is passed between the upper forelimb of the foal and its thoracic wall after which the muscular attachments are severed (Jackson 2004).



• **Fig. 14.16** Bilateral foot–nape posture of the foal. The special danger of this malposture is that the digits press against the vaginal roof during straining, with the risk of its rupture and penetration.

Foot–Nape Posture

In the mare the foot–nape posture is usually bilateral (unlike the cow, in which the anatomy of the forelimbs means that it is more likely to be unilateral). Bilateral foot–nape posture is therefore a condition that is peculiar to the horse, which is made possible by the slender head and long limbs of the foal. Mares present with severe, nonproductive straining. Vaginal examination reveals the fetal head in a normal extended position in the vagina, but the forefeet are found resting on the nape of the neck or on the fetal head. The forelegs may be crossed on the nape of the neck (Fig. 14.16). There is a significant risk of the feet becoming impacted in the dorsum of the vagina, possibly rupturing it and even penetrating the rectum (Benesch & Wright 1951, Jackson 2004). The upper forelimb is identified; then, as the head is repelled and raised, the foot is lifted from its position across the fetal neck and pulled to the appropriate side. The other foot is similarly manipulated and, finally, the head is again raised and each foreleg placed underneath it. Traction is then applied to the head and both forelimbs.

Penetration of the vaginal wall is a common consequence of this malposture. Sometimes the foot penetrates both the vagina and rectum, resulting in a rectovaginal fistula, and, if straining continues when the foot is in the rectum, the entire rectovaginal shelf and anal sphincter can be torn, resulting in a third degree perineal laceration. When one foot is already protruding from the ruptured perineum or rectum, it is preferable to incise the perineum (rather than subjecting it to uncontrolled tearing), deliver the fetus, and then repair both the lacerated and the incised tissue. If repositioning is unsuccessful and the fetus is dead, amputation

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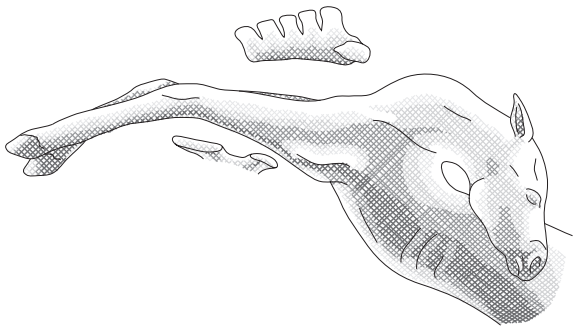
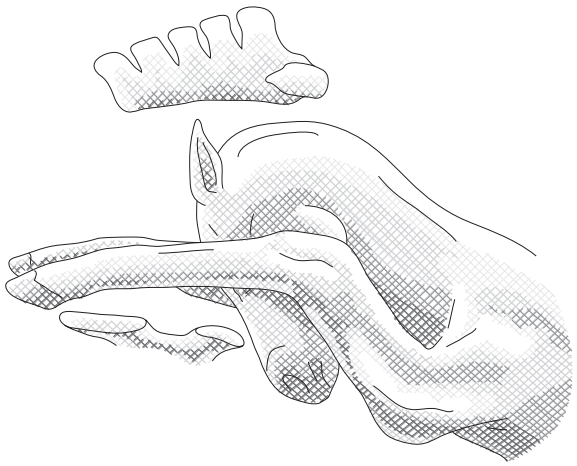


• **Fig. 14.17** Disposition of the foal: anterior presentation, dorsal position, lateral deviation of the head. The fetal nose lies well towards the foal's stifle.



• **Fig. 14.18** Disposition of the foal: anterior presentation, dorsal position, ventral deviation of the head.

of the fetal head or the upper limb – whichever is easier – could be considered. The upper limb is sectioned through the radius by means of the wire saw, and it should then be possible to replace the other limb under the head; the stump of the radius must be carefully controlled during the final delivery. Removal of the entire limb by percutaneous fetotomy of the shoulder may be an alternative.

Lateral Deviation of the Head

This is a serious malposture that is very difficult to correct by vaginal manipulation. Because of the long neck of the foal, the head is out of reach, with its muzzle situated close to the foal's stifle. The long head and muzzle add to the difficulty of correcting such malpostures (Fig. 14.17), and immediate recourse to Caesarean hysterotomy is often the best option for management. Retention of the head is the most common reason for referral of equine dystocia cases to a veterinary hospital.

The fetal forelegs are in the vagina or protrude through the vulval lips, but the head is not presented. Maternal straining may be intense. Vaginal examination confirms the absence of the fetal head in the pelvis, but the base of the neck is palpable and deviates sharply to the right or left (Jackson 2004). Frazer (2011) also noted that deviation of the head can sometimes occur as a secondary consequence of the foal retracting its head during vaginal manipulation. The expulsive forces of the mare can then push the head ventrally or laterally, resulting in this particular malposture. Other cases of lateral deviation of the neck are due to 'wryneck', in which there is a congenital curvature of the cervical vertebrae that cannot be corrected by manipulation (Vandeplassche 1987).

The deviated head is generally beyond the reach of the obstetrician. If the muzzle can be reached, traction can be applied, either by hand or by means of a rope snare. When a snare is used, the internal hand repels the rest of the head and neck. Alternatively, a rope or snare can be placed around the neck to draw the head within reach. Other methods of delivery require the use of special instruments to try to retrieve the head: Kühn's crutch, Blanchard's long flexible hook, and the Krey–Schottler double hook. Their use requires considerable skill and caution (Frazer 2011), and with the availability of safer general anaesthetic methods, they are rarely used these days.

In cases of 'wryneck', when it is quite impossible to extend the neck, the head and neck must be amputated by means of the wire saw fetotome (see Chapter 15), or a Caesarean hysterotomy must be performed (see Chapter 16).

Ventral Deviation of the Head

Downward deviation of the head is less rare in horses than in cattle; nape posture is the most likely to be encountered. The position of the head varies from just being tucked between the forelimbs through to the mandible resting on the sternum (Fig. 14.18). Extension of the head in a mild case can be achieved by repelling the fetus and lifting the muzzle (Jackson 2004). More severe cases can be managed by the application of a mandibular snare: with firm pressure placed upon the fetal brow with one hand, the snare is gently pulled upwards and backwards. The most severe ventral deviations may require the fetus to be deeply repelled and one or more forelimbs to be placed into carpal flexion. Frazer (2011) noted that ventral deviation of the head and bilateral carpal flexion occurs when the fetus has died at the time of first stage labour. If this occurs, the head should be extended before the carpal flexions are corrected. If manipulation does not resolve the dystocia in a reasonable period of time, Caesarean hysterotomy is indicated.

In view of the fact that spontaneous delivery of a foal in nape posture has been observed, it has been suggested that, when the head has projected so far into the vagina that the ears are visible at the vulva, successful delivery by traction without repositioning has occurred; however, this approach is not advisable.

In obstinate cases of nape posture with impaction of a dead fetus at the pelvic brim, fetotomy may be considered. However, the introduction and correct placing of the wire between the markedly flexed head and the neck appears to be very difficult.

Postural Defects of Anterior Presentation in Sheep and Goats

Postural defects are common causes of dystocia in ewes and goat does. The methods of correction are virtually identical in the two species, except that goats tend to resent physical restraint more. When affected sheep and goats are promptly presented, correction is relatively simple and in many cases is successfully carried out by the shepherd or herdsperson. In the following description of the procedures, when reference is made to sheep or ewe, readers can assume that it is the same for does unless otherwise stated. Manipulation is more difficult in the case of large singletons, but delay in providing obstetrical aid is the most frequent cause of difficulty. Repeated ineffectual maternal expulsive efforts cause expulsion of

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the fetal fluids, impaction of the fetus, and close envelopment of the fetus by the uterus. Secondary uterine inertia supervenes and, in protracted cases, the lamb dies and undergoes emphysematous decomposition. Thus even a simple postural defect in such a situation may be very difficult to correct. The veterinarian is likely to see the more serious cases of postural abnormality in which there has been considerable delay and in which unskillful attempts at correction may have caused damage to the ewe's birth canal.

Gentleness of manipulation within the ovine genital tract is most essential; otherwise, serious contusion or laceration of the vagina, cervix, and uterus may result, which is especially liable to be followed by fatal shock or infection. The wool should be clipped from around the perineum and tail base, and this area should be thoroughly washed. Except for the simplest of manipulative procedures, caudal epidural anaesthesia should always be used. The ewe should then be placed in lateral or dorsal recumbency on a hay or straw bale or, if possible, on a table, with the hindquarters overhanging one end. Alternatively, the ewe may be gently restrained when standing or held by an assistant, so that its head and neck rest on the floor, as the hindquarters are raised by grasping the hindlegs above the hocks. The assistant straddles the ewe and maintains the animal in the supine position with her hindquarters at a convenient height for the operator. Fetal fluid supplements, particularly cellulose-based obstetrical lubricants as substitutes for amniotic fluid, should be infused into the uterus. With the advantages provided by raising the ewe's hindquarters and instilling fluid, the majority of postural defects will be readily overcome. The principles of repositioning adopted for the various postural aberrations are the same as those used for the cow. Many cases can be rectified by the hand alone, but snares (including the simple head snare shown in Figure 11.9) may sometimes be useful.

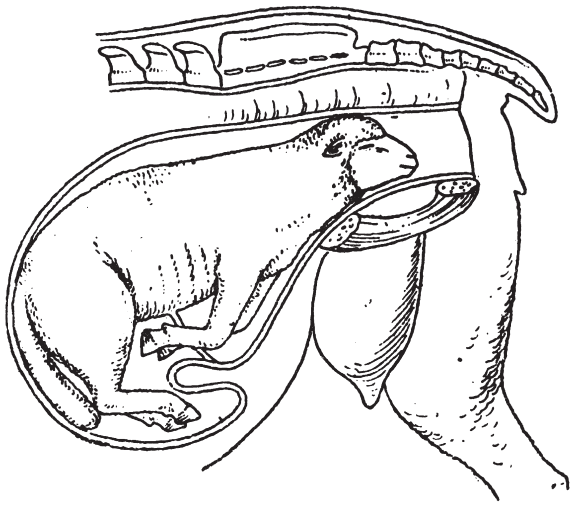
Carpal Flexion Posture

With the ewe held as previously described and with the instillation of fluid in delayed cases, retropulsion is easily achieved. A finger is crooked around the carpus to straighten the leg and the hand cups the foot to protect the uterus. The leg is then grasped by the fetlock to pull the limb clear of the vulva. The ewe is then lowered on to her side and gentle traction is applied each time she strains (Winter & Hill 2003). After delivery of the fetus, the uterus is inspected for the presence of another lamb. Owing to uterine inertia, a second (or third) lamb may fail to be advanced to the pelvic brim. The obstetrician should therefore bring the fore or hind extremity into the pelvis; expulsive efforts of the ewe should then recommence and gentle traction is applied to help delivery.

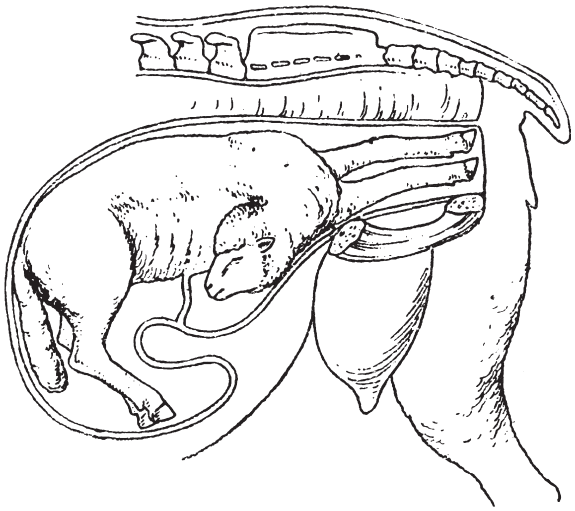
When there has been gross delay and it is found to be impossible to extend the leg of an emphysematous fetus, the carpal joint should be sawn through with fetotomy wire. Copious lubrication is indicated and further fetotomy may be required as described for fetomaternal disproportion. As an alternative, unrelieved carpal flexion may be dealt with by Caesarean hysterotomy (see Chapters 15 and 16).

Shoulder Flexion Posture

Cases of unilateral shoulder flexion that are seen early and in which there is still adequate natural lubrication can be delivered without correction, provided there is no fetomaternal disproportion as well. Winter and Hill (2003) recommend that the ewe is placed in lateral recumbency with the retained limb uppermost, as the limb is more likely to become jammed against the pelvis if it is on the underside. When this is not possible or when both forelimbs are flexed (Fig. 14.19), the ewe's hindquarters should be raised, the fetus repelled, and replacement of fetal fluid provided as necessary. It is then usually possible to reach the lamb's radius/ulna (forearm) and to convert the defect to carpal flexion posture and then to proceed as previously described. It may be necessary to repel the visible leg in order to reach the retained one. In the case of a grossly oversized fetus, when it is found to be impossible to advance the leg, Caesarean hysterotomy may be necessary. When the fetus is emphysematous, the retained limb may be amputated using a wire saw fetotome. After the removal of one limb it is usually possible to deliver the fetus.



• **Fig. 14.19** Disposition of the lamb: anterior presentation, dorsal position, bilateral shoulder flexion posture.



• **Fig. 14.20** Disposition of the lamb: anterior presentation, dorsal position, lateral deviation of the head.

Deviation of the Head

Lateral deviation of the head (Fig. 14.20) is a very common cause of ovine dystocia. As in the cow, it presents with both forelimbs in the vulva, but the head is not in the birth canal. It is important before starting to correct the malposture to ensure that the limbs

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are indeed forelimbs and that they both belong to the same fetus. The head can be deviated laterally (which is the more common postural defect) or ventrally. The methods used for correcting such deviations are similar to those described for cattle. Fresh cases can be managed by repelling the fetus and simple manual repositioning of the head. Longer standing cases or those that cannot be managed by simple manipulation can be managed by elevating the hindquarters of the ewe, together with caudal epidural anaesthesia and the instillation of lubricant as required. Winter and Hill (2003) suggested that the hindquarters should be only partially raised, so that the lamb's head does not fall too far back into the abdomen. The forelimbs can then be repelled and flexed to create space for repositioning the head. A snare may help to straighten the head, or the mandible can be cupped in the hand. Once the head has been corrected, it is advisable to attach a head snare before delivering the lamb by traction. When there is insufficient room to correct the deviation, the displaced head of an emphysematous lamb may be amputated with a wire saw fetotome, but a Caesarean hysterotomy may be preferred.

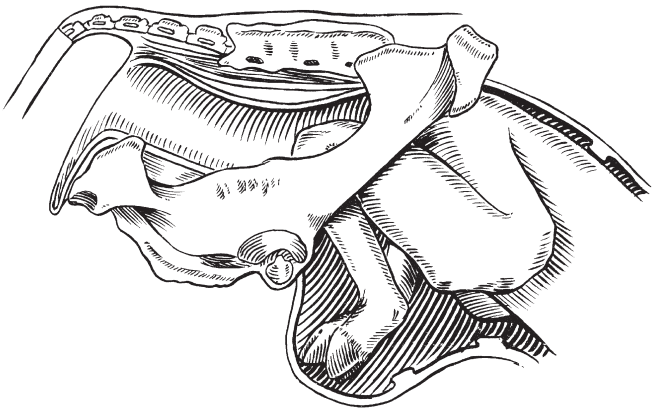
Dystocia Due to Postural Defects: Posterior Presentation

Faulty posture of the hindlimbs is more difficult to correct than abnormalities of the forelimbs, particularly in horses. The defects now to be considered concern the lack of extension of the hock and hip joints, which may affect one or both limbs. In calves it is occasionally found that the umbilical cord runs between the hindlimbs and over the posterior aspect of one or other. In this case it is necessary to create a hock flexion in order to replace the cord in its correct position. Failure to do this may result in its occlusion, causing death of the calf. Owing to the difficulty of extending the retained limbs, due to lack of space in front of the pelvis, there are three essential requirements in attempting to correct this cause of the dystocia: epidural anaesthesia, fetal fluid supplementation, and retropulsion. All manipulations should be conducted very carefully and gently because the danger of accidental perforation of the uterus is a real one. The variable factor exerting the greatest influence on the relative difficulty of the corrective procedure – as well as on the outcome of the operation – is the duration of dystocia before treatment. Cases attended in early second stage labour may be delivered quite easily, but when there has been considerable delay, with consequent loss of fetal fluid, uterine contraction, and death of the fetus, a most difficult and protracted fetotomy or Caesarean hysterotomy may be necessary. A large proportion of stillbirths are associated with fetuses in posterior presentation.

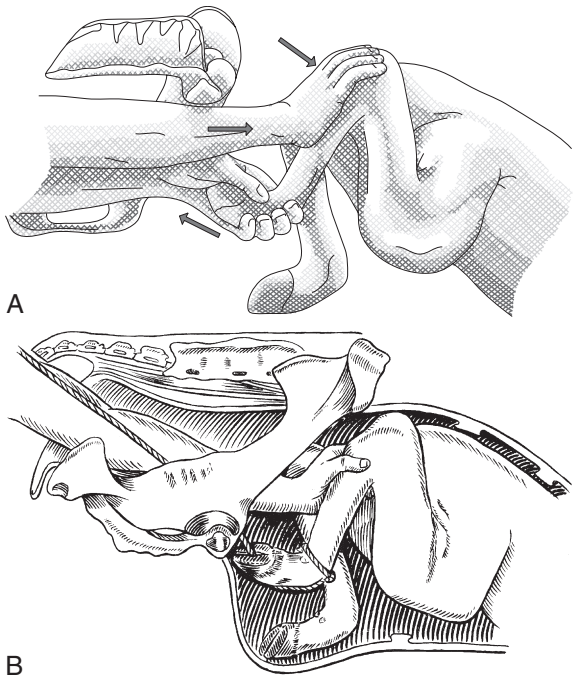
Postural Defects of Posterior Presentation in Cattle

Hock Flexion Posture

The condition is commonly bilateral (Fig. 14.21). The points of the hock may be felt in front of the pelvic brim or may be firmly engaged in the maternal birth canal. An estimate needs to be made of the likely degree of difficulty in correction, and a decision made on whether epidural anaesthesia and/or fetal fluid replacements will be needed. The aim of the manipulative procedure is to extend the hock joint(s), for which enough space must be created. In early cases, with or without epidural anaesthesia, the posture may be corrected by hand. The fetus is first repelled by pressing forward



• **Fig. 14.21** Disposition of the calf: posterior presentation, dorsal position, bilateral hock flexion posture.



• **Fig. 14.22** Correction of hock flexion in the calf. (A) Manual correction by medial and posterior movement of the foot with one hand and lateral, forward, upward repulsion of the hock with the other hand. (B) Correction of hock flexion using the hand and a digital snare.

on its perineum. The foot is flexed in a cupped hand and drawn backwards and medially as, with the other hand, the point of the hock is grasped and pushed upwards, forwards and laterally (Fig. 14.22A). The foot is eventually lifted over the pelvic brim, and the limb is extended in the vagina. In cases in which it is found to be impossible to extend the hock owing to the lack of space, an assistant is directed to pass in an arm and to press forwards and upwards on the point of the hock as the operator again tries, as before, to bring the foot into the pelvic canal. An alternative method is to supplement manual extension by traction on a snare fixed to the retained foot in this way: One end of an obstetrical snare – to which may be attached Schriever's introducer – is passed into the birth canal, around the hock flexure, brought out, and passed through the loop at the other end; the running noose thus formed is applied to the metatarsus. The noose is then manipulated

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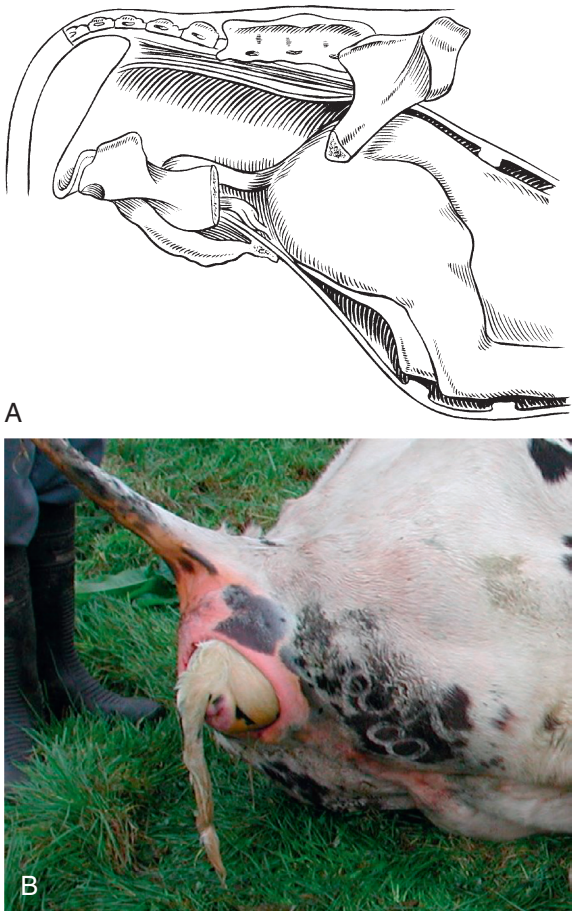

• **Fig. 14.23** (A) Disposition of the calf: posterior presentation, dorsal position, bilateral hip flexion posture ('breech presentation'). (B) Clinical appearance of breech presentation, with the tail protruding from the vulva. (B: Courtesy Dr. Jakob Malmo.)

down the limb until it lies in the pastern, the shank of the noose being placed between the digits, so that when traction is applied to it the fetlock and pastern, joints are flexed (see Fig. 14.22B). After again repelling the fetus the obstetrician grasps the foot and, as the assistant pulls on the snare, the extremity is lifted over the pelvic brim.

In the occasional case in which it is impossible to extend the hock and the calf is dead, simple fetotomy may be performed (see Chapter 15). If the calf is alive, then a Caesarean hysterotomy will be necessary.

Hip Flexion Posture and Breech Presentation

Bilateral hip flexion is more common in cattle than unilateral flexion. When both hips are flexed, the condition is described as a 'breech presentation' (Fig. 14.23A). The calf's tail is presented, either through the vulva (see Fig. 14.23B) or palpated on vaginal examination. The difficulty of the dystocia depends on how long standing it is: fresh cases are relatively easy to manage, but neglected cases that are heavily impacted in the pelvis or in which a hand cannot be passed to the hocks of the calf can be very difficult to manage. Many cases of breech presentation occur in twin calvings, with the benefit that the calf may be smaller than if it were a singleton. However, the co-twin is often also in posterior presentation.

The aim of the treatment is to convert the breech presentation into a bilateral hock flexion and then proceed accordingly. Most cases of breech presentation will require epidural anaesthesia unless the calf is very small. Some will also require fetal fluid replacement. The manipulative procedure is to repel the calf's perineum forwards and upwards with the aim of bringing the retained limbs within reach, so that the leg may be grasped as near to the hock as possible. Traction on the limb converts the posture into hock flexion, from which point the hock is extended as described previously. If it is impossible to bring the hock within reach, a fetotomy should be performed if the calf is dead, or Caesarean hysterotomy if it is alive.

In cases of a small fetus, delivery may be attempted with the fetus still in the breech presentation. Snares are introduced between the upper limbs and the pelvis of the fetus and then picked up from below. Using a Schriever's introducer will make this procedure a lot easier. Subsequently, the fetus can be delivered by traction on both snares.

Postural Defects of Posterior Presentation in Horses

Uncomplicated Posterior Presentation

Posterior presentation in the mare carries with it an increased risk of fetal hypoxia, due to compression of the umbilical cord in the birth canal, premature rupture of the umbilical cord, or rupture of the cord while the head of the foal is still in the uterus. Uncomplicated posterior presentation can be managed by gentle traction. However, a high proportion of foals in posterior presentation also have abnormalities of posture or position, probably due to compromise of the normal process of rotation and extension of the extremities (Jeffcott & Rosdale 1979). Hence foals in posterior presentation appear to be predisposed to being in a dorsoilial position (Frazer et al. 1997) or to have bilateral malpostures of the hindlimbs (Frazer 2011).

Hock Flexion Posture

Hock flexion is much more difficult to manage in the mare than in the cow, owing to the very long limbs of the foal. Hence many cases have to be managed by Caesarean hysterotomy or fetotomy rather than vaginal manipulation. Bilateral hock flexion may present with the tail present at the vulva and the flexed hocks at the pelvic inlet. Unilateral flexion will present with a single hindlimb protruding through the vulva (Jackson 2004).

In principle, the methods used for correcting hock flexion are like those described for cattle, but, owing to the longer limbs of the foal, the procedure is much more difficult. For manipulation, the fetus has to be repelled well forwards, a process that is likely to require fetal fluid substitution and/or the administration of a tocolytic drug. An assistant may help to repel the fetus, although there are risks to the safety of personnel when two people are working closely behind the mare. Even so, repelling the fetus may only be possible in the anaesthetised mare with her hindquarters elevated. Straightening the hock is more risky than in the cow, as the hock will be forced against the dorsal wall of the uterus, and there is further risk to the floor of the uterus from the foal's hoof as it is difficult to reach the foot to cup it in the hand (Frazer 2011). Use of a crutch to repel/elevate the hock and of a snare to provide traction on the distal limb has been described. If the foal is alive, it may be preferable for an early recourse to a Caesarean hysterotomy; if it is dead, fetotomy may be the best means of resolving the malposture (Embertson 1992, 1999, Frazer et al. 1997).

Hip Flexion Posture and Breech Presentation

Correction of hip flexion posture, especially breech presentation (Fig. 14.24), is difficult. In addition to the difficulties of managing

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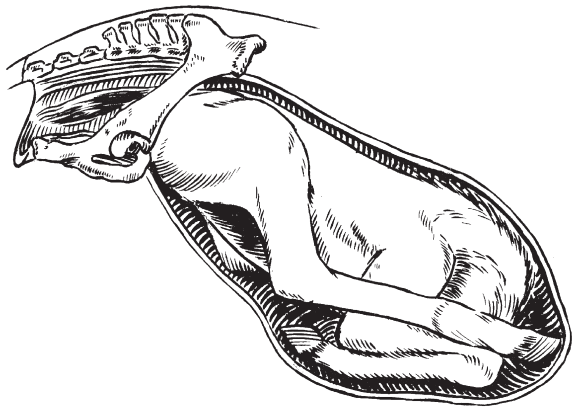
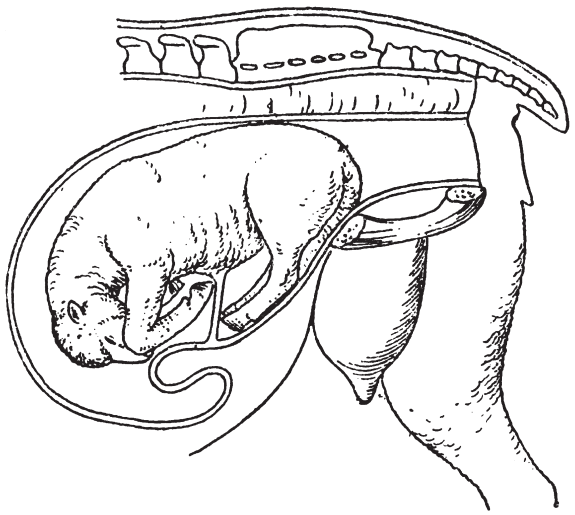


• **Fig. 14.24** Disposition of the foal: bilateral hip flexion posture ('breech presentation').



• **Fig. 14.25** Disposition of the lamb: posterior presentation, dorsal position, bilateral hip flexion posture ('breech presentation').

hock flexion (see previous mention), each limb has first to be converted from hip flexion to hock flexion. As in cow, hip flexion is converted into hock flexion by repelling the fetus, grasping the hock, and drawing it backwards towards the pelvic inlet. Also, as in the cow, a bilateral hip flexion should be converted into a bilateral hock flexion before attempting to extend either hock. There is a very real danger of trauma to the uterus and birth canal during these procedures. There may be benefit in anaesthetising the mare and placing her with the hindquarters elevated. However, if after a proper effort, attempts to extend the hindlimbs are unsuccessful and the foal is still alive, no time should be lost before resorting to Caesarean hysterotomy. If, as is more likely, the foal is dead, then under general anaesthesia and after amputation of one or both hindlimbs by means of the wire saw fetotome, it should be possible to deliver the foal by traction. Frazer (2011) recommended converting a breech presentation into a bilateral hock posture before cutting both limbs through the distal tarsal bones.

Postural Defects of Posterior Presentation in Ewes and Does

A considerable proportion of twin lambs and kids are in posterior presentation, and because of the lack of uterine space, especially when both lambs occupy one uterine horn, one or both hindlegs may fail to extend into the vagina (Fig. 14.25). Thus, in flocks with a high proportion of twins, hock flexion and hip flexion postures will be common causes of dystocia.

These malpostures may be corrected in the manner described for cattle but, because twin lambs are smaller than singles and because it is a simple matter to raise the ewe's hindquarters, the requisite manoeuvres are much more easily performed than in cattle. In all delayed cases, fetal fluid substitution is indicated. The manipulation of the fetus, including its retropulsion, should be carried out in a very gentle manner. In cases of irreducible malposture in dead lambs, appropriate fetotomy or Caesarean hysterotomy can be performed.

Dystocia Due to Faulty Position

Faulty position of the fetus is encountered more frequently in horses than in ruminants. This is considered to be due to the fact that, in late gestation or first stage labour in horses (but not in cattle), a physiological rotation of the fetus from the ventral to the dorsal position occurs; however, occasionally this turning fails. In this case the fetus is in an anterior presentation (occasionally posterior) but with its vertebral column applied to either one side of the uterus (right or left lateral position × 2 dorsoilial position) or facing the floor of the birth canal (ventral position × 2 dorsopubic position). The process whereby the ruminant fetus sometimes comes to lie in ventral position is not understood. It is unlikely to be a gestational position; more probably it arises during the first stage of labour, when the uterine peristaltic force generates a vigorous reflex response in the fetus that causes it to rotate about its longitudinal axis.

In order to make birth possible, fetuses in lateral or ventral position must be rotated into the normal upright (dorsosacral) position. This can be achieved by first repelling the fetus and then rotating it by appropriate force applied to the presenting extremity. Such rotation is easier to perform with the patient standing. Epidural anaesthesia is useful for this manoeuvre.

Anterior Presentation/Lateral Position in the Cow and Horse

These abnormalities of position are best managed in the standing position. If there is a concurrent malposture, it is generally best to correct this first, before correcting the malposition.

In the case of a live calf or foal, it may be possible to correct a lateral position by means of evoking a convulsive reflex by the fetus with pressure from the thumb and middle finger on the fetal eyeballs (the latter being protected by the eyelids). By applying a rotational force in the appropriate direction, the fetus can be turned into the dorsal position. The fetal nose and forelimbs are then advanced into the maternal pelvis, and the maternal expulsive efforts are assisted by grasping both forelimbs and gently pulling on them and, at the same time, applying a rotational force.

Alternatively, snares can be applied to the head and both forelimbs. One hand is advanced under the thorax on the side of the ventrally placed limb, grasps the shoulder joint, and moves it upwards and in a medial direction. Simultaneously, traction is placed on the upper limb in the opposite directions (e.g., downwards and left for a right lateral position). Another approach is that rotation is performed by repelling the fetus as far cranially as possible, crossing the snares in the appropriate direction, and

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then applying traction. Such a force tends to result in the snares becoming more or less parallel, which can only occur if the fetus rotates about its longitudinal axis. It is important to ensure that the snares are crossed in the right direction so that rotation of the fetus is not increased. The use of detorsion rods for correction of abnormalities of position has also been described. For all of the methods described to be effective, epidural anaesthesia and fetal fluid substitution are likely to be necessary.

Anterior Presentation/Ventral Position in the Horse and Cow

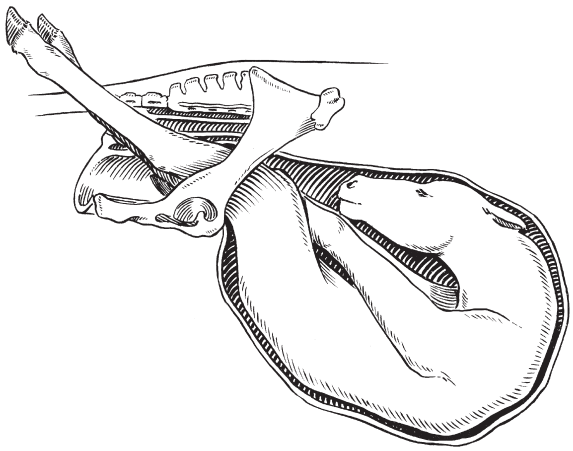
The same methods as described previously for the correction of a lateral position defect can be used, although the procedures will usually need to be repeated several times. Placing the dam with the hindquarters raised will facilitate the procedure. If the calf or foal should rest on its back with the head and limbs flexed on to its neck and thorax, the fetus must first be repelled so that the head and forelimbs can be extended. Rotation is then carried out.

Posterior Presentation/Lateral or Ventral Position in the Horse and Cow

Lateral presentation can be managed by the veterinarian introducing a hand and grasping the stifle region of the upper limb. Simultaneous retropulsion and downward pressure are applied to rotate the fetus through 90 degrees.

Ventral position can be managed by the veterinarian introducing a hand between the fetal hindlimbs and up to the inguinal region, where one of the thighs is grasped; then, pushing forwards, the operator rotates the fetus through a half circle. Failing this, traction on crossed limb snares should be used. If the hindlimbs are protruding far enough from the vulva, an alternative procedure is to place a traction bar (a length of broom handle will suffice if a specific instrument is unavailable) between the projecting hindlimbs and to secure it to them by means of a snare; rotational force is then applied to the traction bar.

There is a grave risk that the hind feet of a foal in posterior presentation/ventral position will penetrate the vagina and rectum (Fig. 14.26). In such a case a Caesarean hysterotomy should be performed and the rectovaginal fistula repaired later.



• **Fig. 14.26** Disposition of the foal: posterior presentation, ventral position, extended posture; penetration of the dorsal wall of the vagina and dorsal wall of the rectum, resulting in a rectovaginal rupture.

Dystocia Due to Defects of Position in Sheep and Goats

The methods of treatment are those described for the horse and cattle. By raising the ewe into the inclined supine position and infusing fetal fluid substitute, rotation is much easier in this species; instruments are seldom required.

Dystocia Due to Faults of Presentation

Instead of the long axis of the fetus being in line with the birth canal, it may be disposed vertically or transversely to the pelvic inlet. Owing to limitation of space in the sagittal plane, absolute vertical presentation is not possible, but oblique vertical presentations do occur - rarely, and then more often in mares than cows. Vertical presentations are described according to whether the fetal vertebral column or abdomen is presented at the pelvic inlet, i.e., dorsovertical or ventrovertical presentations. Transverse presentations are even more uncommon and are almost exclusively encountered in the mare; they may be ventrotransverse or dorsotransverse. Finally, there is the bicornual transverse presentation of the mare, in which the limbs of the fetus are positioned in the uterine horns rather than in the uterine body. All of these faults of presentation are difficult to manage, the bicornual presentation especially so. When correction of the malpresentation is possible by vaginal manipulation, it is achieved by version of the fetus into a longitudinal presentation. In principle the nearer extremity is moved towards the pelvic inlet, although it is simpler to convert to a posterior than an anterior presentation (i.e., two appendages being manipulated rather than three). Many cases cannot be delivered by manipulation, so resort often needs to be made to fetotomy, hysterotomy, or manipulation via a laparotomy. Transversely presented foals are often larger than normal, as the placenta fully occupies both uterine horns, which adversely affects the prospects for vaginal delivery. Likewise, dystocia due to transverse presentation may be associated with weak signs of labour: because the fetus does not engage into the pelvis, abdominal straining may be minimal. Hence, the signs of labour may not be recognised.

Oblique Dorsovertical Presentation in the Horse

This is the rarest of the malpresentations, with occurrence largely restricted to the mare. In the dorsovertical presentation, the fetus lies with its back or withers directed towards the pelvic inlet whereas the body of the fetus is 'lying' in the uterine body. Benesch and Wright (1951) were of the opinion that this presentation occurs when premature rupture of the fetal membranes occurs before fetal extension and rotation has occurred. The presentation is extremely difficult to resolve because no fetal limbs are accessible.

Vaginal delivery has been described, but would be very difficult indeed to carry out successfully. The process is that, according to whether the head or breech is nearer the pelvic inlet, conversion into anterior or posterior longitudinal presentation may be attempted. An attempt is made to bring the fetal extremity (head and/or limbs) to the pelvic inlet and first to convert the defect into a ventral longitudinal presentation. The fetus can then be rotated to the dorsal position as described earlier. Retropulsion and the presence of copious fluid (natural or artificial) in the uterus are both essential. A grip is taken on the fetus by means of Krey's hook as near as possible to the more proximal fetal extremity. Then, as retropulsion is applied, the hook is pulled on with a view to bringing the fore or hind end of the fetus to the pelvic

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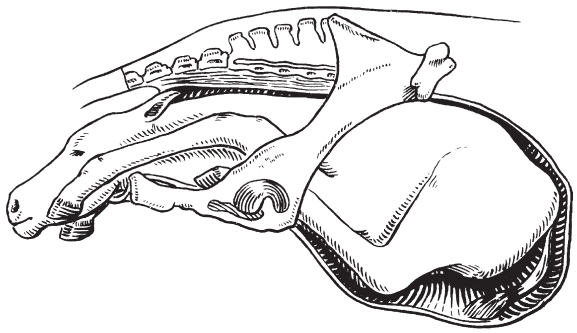

• **Fig. 14.27** Disposition of the foal: oblique ventrovertical presentation ('dog-sitting position'). Note the presence of the hind feet in the pelvic canal.

inlet. After adjustments of position and posture, the fetus is then delivered by traction.

In principle, if manipulation is not possible, it should be possible to resolve the case by fetotomy or Caesarean hysterotomy but reports of such management are exceedingly rare. Benesch and Wright (1951) suggested that fetotomy should be undertaken as though the case were a dorsotransverse presentation.

Oblique Ventrovertical Presentation in the Horse and Cow ('Dog Sitting' Position)

This presentation is less rare than the dorsovertical position, but is still very uncommon. Its occurrence is largely restricted to the mare, although occasional cases occur in ruminants. In the 'dog sitting position' the fetal head, neck, and forelimbs are presented in the vagina, but, as one or (usually) both hips are flexed, the distal parts of the hindlimbs are also in the birth canal or pelvic inlet (Fig. 14.27). Sometimes, only one hindlimb is flexed (which has been called a 'hurdling' position). Frazer et al. (1997) considered that the unilateral posture is more common in mares than the bilateral.

The initial stages of birth appear normal, with the head and forelimbs presented at the vulva. Thereafter, despite further straining by the dam or traction by assistants, no further progress is made. Close examination shows that the forelimbs are less far advanced than normal (which also occurs in cases of elbow flexion). Insertion of a hand into the vagina reveals the presence of the hind feet resting on the pelvic floor. Sometimes the hind feet can even be present in the vagina. Because of the co-presentation of the hind feet into the pelvis, further traction on the fetus will cause its greater impaction into the pelvis, as well as increasing the risk of damage to the cervix.

Correction of this malposition is difficult. It may be possible to correct cases that have not become impacted by cupping the feet and pushing them off the pelvic brim as far forward into the uterus as possible. However, the obstetrician's arm is often not long enough to do so, especially in the mare, and there is a significant risk of damage to the uterine floor. Once the hind feet have been repelled, the fetus is delivered by traction.

When the fetus is impacted, which is usually the case, other methods are needed. After epidural anaesthesia and substitution of fetal fluids, Roberts (1986) suggested placing a Kühn's crutch around the pastern joints of the hindlimbs as a means of repelling them into the abdomen. An alternative method has been described, by which the fetus is converted into a posterior presentation/ventral position, then turned into the dorsal position. Benesch and Wright (1951) described placing snares on the hind pasterns and a crutch near the elbows of the forelimbs. The forelimbs are then repelled and the hindlimbs are drawn forwards, after which the hindlimb snares are used to rotate the fetus into the dorsal position. Baldwin et al. (1991) rightly suggested that, if the fetus is dead, repelling the fetus should not be attempted in the standing mare, as the repelled limb is likely to remain flexed, posing a high level of risk of rupture of the uterine wall. In such circumstances, the mare should be anaesthetised and positioned with the hindquarters elevated, before attempting manipulation.

Resolution of this malposition by Caesarean hysterotomy or fetotomy is equally not straightforward. Caesarean hysterotomy will be difficult unless the fetus can be repelled into the uterus; Jackson (2004) suggested that laparotomy and hysterotomy may be a preferable alternative approach. A relatively small incision is made in the uterus, through which the malpositioned limbs are detached from the pelvis and extended back into the uterus. The fetus is thereafter delivered *per vaginam* by traction, and the uterine incision closed.

Fetotomy is complicated and beyond the capabilities of all but the most experienced practitioners. A transverse cut is made through the trunk, the fetus eviscerated, and the lumbar vertebrae removed close to the pelvis. After applying snares to the hindlimbs, the fetus is repelled into posterior presentation and delivered by traction. Alternatively, one forelimb can be removed, costochondral junctions cut, and the fetus eviscerated. The pelvis is then split to remove one hindlimb and the tail, and the segments of the fetus delivered (Baldwin et al. 1991). If the fetus has been drawn so far into the birth canal that the head and shoulders are through the pelvis, fetotomy is the only recourse for delivery.

Dorsotransverse Presentation in the Horse and Cow

This is an extremely rare cause of dystocia (Fig. 14.28A and B), in which the spine of the fetus is presented towards the birth canal. Oblique variants of it also occur in both the mare and cow. Delivery by manipulation is difficult or, in many cases, impossible. Hence, the preferred method of delivery is by Caesarean hysterotomy, regardless of whether the foal is alive or dead (Vandeplassche 1987).

If manipulation is attempted, replacement fetal fluids should be instilled and the mare should be anaesthetised and placed on her back; a cow should be given epidural anaesthesia. The obstetrician should ascertain whether one extremity is nearer the pelvic inlet. The technique of correction required involves repulsion of the fetus and the advancement of its nearer extremity to the birth canal; so, unless one extremity is within easy reach, uterine version is likely to be extremely difficult or impossible. An attempt is made by manipulation of the proximal fetal extremity to turn the fetus into ventral position, anterior or posterior presentation. The next step is to rotate the fetus into dorsal position. Finally, it is delivered by traction. If after a short, determined effort, it is obvious that version cannot be achieved, Caesarean hysterotomy should be performed immediately. Fetotomy is very difficult to carry out in this type of dystocia and, consequently, is not recommended (Vandeplassche 1987).

Ventrotransverse Presentation in the Horse and Cow

This presentation, in which the abdomen and limbs of the fetus are presented towards the birth canal (Fig. 14.29), is more likely to be seen in the mare than the cow, and oblique variants of it are

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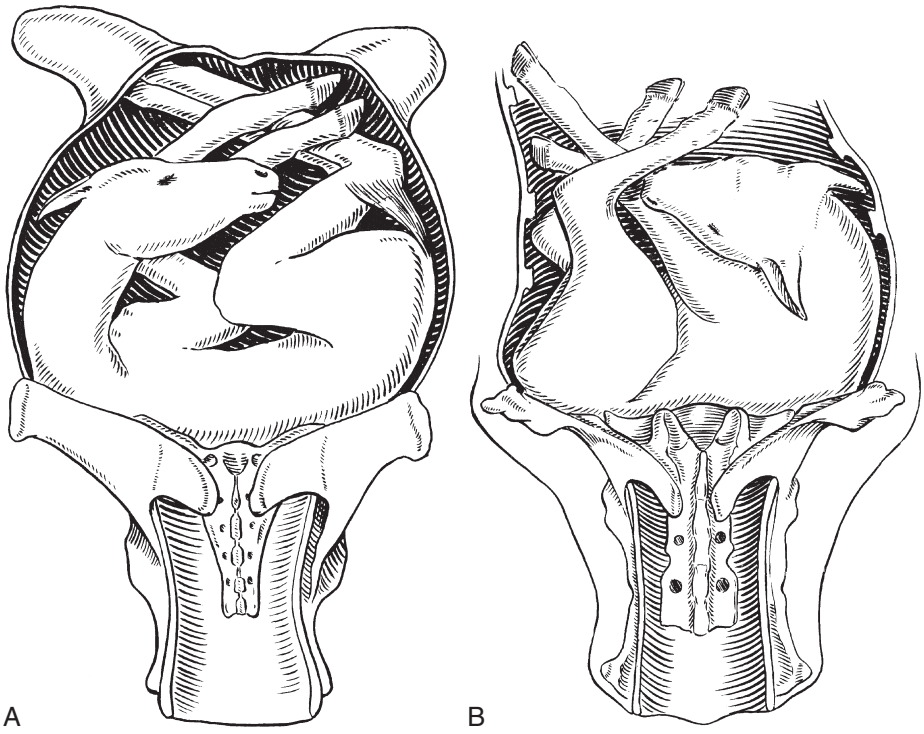
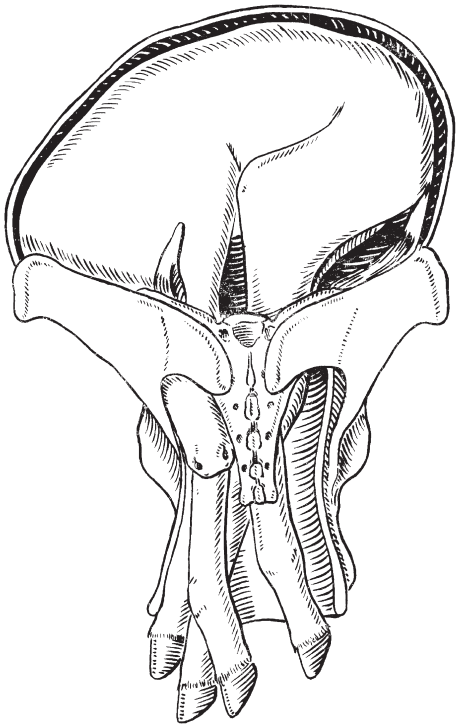


• **Fig. 14.28** (A) Disposition of the foal: dorsotransverse presentation; uterine body gestation. (B) Disposition of the calf: dorsotransverse presentation.



• **Fig. 14.29** Disposition of the foal: ventrotransverse presentation; uterine body gestation.

more usual. A variable number of fetal appendages may enter the maternal pelvis. It is possible that the head as well as the forelimbs are in the vagina, but it is usual for two or more legs only to be presented. The condition must be distinguished from twins and double monsters and from schistosoma reflexus (see Chapter 9).

Fetal abnormalities, such as limb deformities, angulation and/or ankylosis, and vertebral deformities, such as 'wryneck', occur in a significant proportion of such cases. Roberts (1986) believed that this is due to restriction of fetal movement *in utero*.

The method of choice for delivery of a fetus in this position is Caesarean hysterotomy because delivery by vaginal manipulation will be time-consuming and require much experience (Frazer 2011). If attempted, the aim of vaginal interference is first to convert the abnormality into longitudinal – usually posterior – presentation, ventral position; this means that the hindlimbs are extended into the pelvis, as the head and forelimbs are repelled into the abdomen. General anaesthesia and elevation of the hindquarters are helpful in the mare. Unless progress with version is soon apparent, Caesarean hysterotomy is recommended for both mare and cow.

Bicornual Transverse Presentation in the Mare

In the bicornual type of transverse presentation peculiar to mares, the fetal extremities are disposed in the two horns, and its trunk lies across the cranial portion of the uterine body (Fig. 14.30). Ventral displacement of the uterus may have occurred, and if so, it may be impossible to palpate the fetus. As soon as the presentation is recognised, a Caesarean hysterotomy should be performed.

Dystocia Due to Defects of Presentation in Sheep and Goats

The methods of treatment are similar to those described for the mare and cow. By raising the ewe into the inclined, supine position and by infusing fetal fluid substitute, version is much easier in this species, but in protracted dystocia Caesarean hysterotomy may provide an easier solution.

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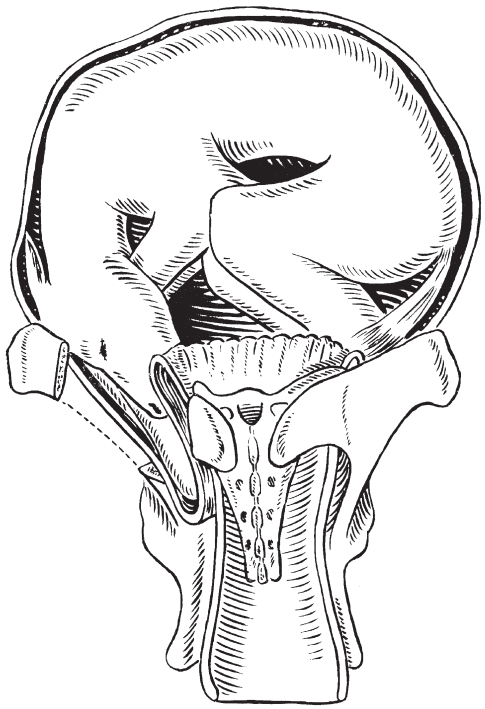
Dystocia Due to Twins

Twin gestation in cattle often culminates in dystocia. Mee et al. (2011) and Olson et al. (2009) respectively determined that the risk of dystocia in dairy cows carrying twins as 2.4 times or 3.7 times that of cows carrying singletons (see also Table 14.2). Twin foaling in mares is uncommon because abortion is a more probable outcome. However, equine twins that survive to term may well cause dystocia: Leidl et al. (1993) reported 4% of referred cases of equine dystocia were due to twins. In sheep and goats the prevalence of maldisposition of fetuses increases with litter size, although the birth weight of the fetuses is reduced. Thus the effect of twins versus singletons is generally that the prevalence of dystocia is reduced, but in litters of three or more lambs, it is increased (Dwyer & Bunger 2012) (see also Chapter 12). In general, the smaller size of twin fetuses facilitates manipulative correction and delivery; for the same reason, natural or obstetrical delivery may be possible despite maldisposition of a fetus.

The relative importance of unilateral or bilateral twin pregnancies to the development of dystocia was studied by Echternkamp et al. (2007) in calves born to cows selected for twin ovulations.



• **Fig. 14.30** Disposition of the foal: ventrotransverse presentation with ventral displacement of the uterus in the mare; bicornual gestation.

They found that the survival at birth of twin calves derived from unilateral pregnancies was lower and the risk of dystocia higher than in calves derived from bilateral pregnancies (Table 14.2). These differences may perhaps be attributed to a greater risk of simultaneous presentation when a twin from each horn approaches the pelvic inlet, but a greater risk of malposture and/or inertia when both fetuses occupied the same horn. Overall, therefore, the risk of stillbirth in twin-born calves is significantly greater than that of singletons: Olson et al. (2009) calculated the odds ratio for stillbirth of twins as 7.8 times that of singletons. It has been suggested that the second-born twin is more likely to survive than the first-born.

Twin dystocia is of three types:

- Both fetuses present simultaneously and become impacted in the maternal pelvis (Fig. 14.31A and B). Fetuses may be both in the same presentation, or one may be anterior and the other posterior.
- One fetus only is presented but cannot be born because of faulty disposition, usually due to failure of normal extension of limbs or head because of insufficient uterine space.
- Uterine inertia due to either overstretching of the uterus by the excessive fetal load or premature birth. When inertia is present, birth of the first or second fetus does not proceed even when the presentation is normal.

In the treatment of twin dystocia, the first essential is to determine which fetal appendage(s) is (are) presented and its (their) relationship to each of the fetuses. Each appendage must be traced back to the trunk to which it attaches before any further action is undertaken. If this is made a rule, the obstetrician will not blunder into applying traction simultaneously to two fetuses. Nor should twins be mistaken for a schistosome, double monster, or ventrotransverse presentation of a single fetus.

When a twin is presented with an abnormality of posture, it is managed as though it were a single fetus; in such cases the presence of twins may not be known – but may be suspected on account of small fetal size and the history of the dam – until the uterus is searched after delivery and another fetus found. Again, the association of uterine inertia with twins may be known only after delivery of the first fetus. Breech presentations are common in ruminant twins, and the second fetus is often also in posterior presentation.

When twins are known to be present and retropulsion is required to allow one fetus an unimpeded assisted exit or to provide space in order to correct a simple malposture, it should be performed very carefully. In cattle, and especially in sheep and goats, there is a much greater likelihood of causing uterine rupture when twins are present. Spontaneous rupture has been observed when both fetuses were in the same horn.

TABLE 14.2 Effect of fetal number and location *in utero* on calf survival at birth, calving assistance and fetal malpresentation

Number and Disposition of Pregnancies	n	Calf Survival at Birth (%)	Calving Assistance (Total) (%)	Malpresentations (%)
Single	1587	97.2	22.5	5.5
Twin, unilateral pregnancy	1284	83.0	57.8	47.4
Twin, bilateral pregnancy	1158	94.0	49.8	33.6

Derived from Echternkamp et al. (2007).

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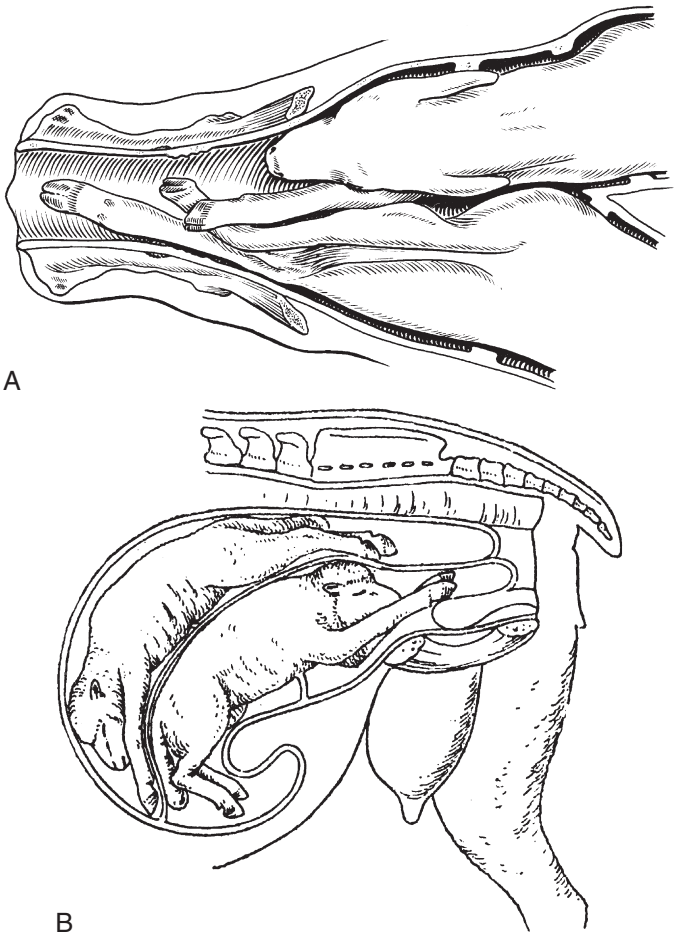

• **Fig. 14.31** Simultaneous engagement of (A) bovine and (B) ovine twins. In each figure, one twin is in anterior presentation, dorsal position, shoulder flexion posture; the other is in posterior presentation, dorsal position, extended posture.

Simultaneous presentation of twins (see Fig. 14.31A and B) is treated in logical sequence. The polarity of the fetuses is determined, the more advanced fetus recognised, and its presenting extremity appropriately snared. Any defect of presentation, position, or posture must be diagnosed and treated; correction may be facilitated by epidural anaesthesia. Then, with continuing retropulsion on the less advanced fetus, the nearer one is brought into the pelvis and delivered by simple traction. The other fetus, which may be presented in the opposite direction, is then appropriately manipulated. If one fetus is in posterior presentation, there may be benefits in delivering it first, as there are only two appendages to manage. The delivery of ovine twins is more easily achieved if an assistant holds the ewe by its hindlegs in an inclined, supine position. When the ewe is delivered of twins, the uterus should always be examined for another fetus(es).

In occasional cases of prolonged delay, corrective manipulation is impossible, and fetotomy of the presenting fetus may be required. Severe pelvic impaction of dead fetuses may be more readily relieved by Caesarean hysterotomy.

Retention of the fetal membranes is more common in cows with twin births than singleton births (Bell & Roberts 2007). In sheep the risk of retained fetal membranes is only increased if there is also dystocia.

Most cases of equine twin conception are followed by early death of one or both of the conceptuses (see Chapter 31). About 2% of equine gestations start as normal twins, but mummification or abortion frequently occurs, so that less than 1% reach term. Vandeplassche et al. (1970) reported data from 44 cases of equine twin gestation. All pairs were of dizygotic origin (i.e., nonidentical). In 33 of 34 twin pregnancies, it was found that one fetus occupied each horn; in the remaining cases the twins were in the same horn. Of 44 live foals born, 37 were reared. The study showed that there was a much smaller likelihood of viable twin foals being born to thoroughbred mares than to Belgian draught mares, and this difference might be related to a better uterine capacity in the draught mare (Vandeplassche et al. 1970). Retention of fetal membranes is likely to occur after twin foal births (Threlfall 2011), although possibly only if there has also been concurrent dystocia (Provencher et al. 1988).

Dystocia Due to Fetomaternal Disproportion

Fetomaternal Disproportion in Cattle

Fetomaternal disproportion is the most common cause of dystocia in cattle, particularly in primiparous animals. Its aetiology and prevalence are discussed in Chapter 12. Although the precise aetiology may be of an oversized calf or an undersized dam, the clinical signs and management are essentially identical. The clinical signs are of a cow that is unable to complete second stage labour, despite straining unproductively for a time in excess of the normal duration of second stage labour (i.e., about 2 hours for a multiparous cow). The fetus is in a normal disposition and the allantochorion ('water bag') has ruptured. If in anterior presentation, the two forefeet are visible at the vulva, often accompanied by the fetal nose. Many cases of fetomaternal disproportion do not receive timely veterinary intervention; often, when the veterinarian arrives, the animal has been in second stage labour for several hours, and there is a certain degree of secondary uterine inertia present.

The first main point of difficulty is associated with the birth of the fetal head. In heifers this can be due to a failure of the posterior vagina and vulva to adequately dilate. The next point of difficulty is associated with the entrance of the fetal chest and shoulders into the birth canal. The shoulders are normally the greatest presenting diameter of the fetus, so the greatest effort is typically expended in delivering this part of the fetus.The hips are the final main point of difficulty. If the gestation was prolonged, the relatively high growth rate of the fetal pelvis can result in the hips becoming 'locked' within the maternal pelvis.

Some cases of apparent fetomaternal disproportion occur when the fetus has died (usually during a neglected labour), and it has become decomposed and emphysematous. The presence of fetal emphysema results in a fetus whose diameter is too great to pass through the birth canal.

Fetomaternal disproportion can be managed in one of four main ways:

- The normal expulsive forces may be supplemented by external traction on the fetus. This method is frequently employed successfully by stock managers.
- The diameter of the vulval opening may be increased by episiotomy.
- The fetus may be removed by Caesarean hysterotomy.
- The overall size of the fetus may be reduced by fetotomy. The use of fetotomy is generally restricted to when the fetus is already dead.

Assessment of which of these methods to use will be guided by the degree of disproportion, the duration of the dystocia, whether or

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not the calf is alive, and the value of the calf. Welfare and survival of dam and fetus and preserving the dam's subsequent fertility are also important considerations.

In the case of a group of animals in which dystocia is being caused by fetomaternal disproportion, consideration should be given to inducing early parturition in the remainder of the group.

Assessment of Disproportion

Assessment of the degree of disproportion is made by observation of the presenting parts and palpation of the fetus within the vagina. No single method provides unequivocal evidence of the degree of disproportion, but, taken together, identification is generally reliable.

Size of the Fetal Limbs. The diameter of the fetlocks are generally a good guide to the size of the calf (Hindson 1978, Ko & Ruble 1990), although there are discrepancies, as in the case of the Belgian Blue calf whose shoulders are much wider than the lower limbs would suggest and in the emphysematous calf whose trunk is massively disproportional to the size of its feet. Hiew et al. (2016) explored the relationship between front foot circumference and various parameters of calving difficulty in Holstein cattle, finding that there was a significant linear relationship between body weight and foot circumference but not between calving difficulty score and foot circumference.

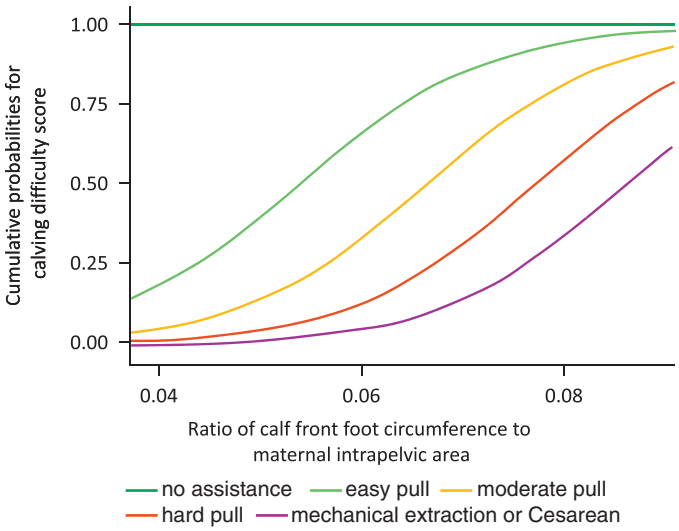
Size of the Head. Palpation of the fetal head (width of poll or muzzle) also gives an indication of the body size of the calf. Colburn et al. (1997) could not find a direct relationship between head circumference of calves and calving difficulty score, although the circumference of calves born to bulls with a low EPD (expected progeny difference) for birth weight was smaller than of those born to bulls with a high EPD. Nugent et al. (1991) likewise found that calves sired by bulls with a high EPD for birth weight had smaller canon bones and a lower head circumference than did those sired by low EPD bulls. In neglected cases of fetomaternal disproportion, the head can become swollen and oedematous, giving a false impression of its size.

Pelvic Area: Ratio of Pelvic Area to Calf Size. The relationship between pelvic area and the risk of fetomaternal disproportion are discussed in Chapter 12. Basic assessment of the relative size of the fetus and the dam's pelvis is made by passing a lubricated hand around the fetus within the pelvis to estimate the amount of space between the fetus and pelvis. If the head is tightly wedged inside the pelvis or if the shoulders appear to be impacted in the pelvic inlet, it is likely that there is a significant degree of disproportion. If the calf is in posterior position, a hand can be used to explore the amount of space between the calf and the dam's pelvis. If the thighs and hips have entered the pelvis, it is likely that the calf can be delivered. If, however, the thighs are not in the pelvis and appear to be wedged at the pelvic inlet, delivery of the calf is less likely.

Hindson (1978) found a significant correlation between the external distance between the medial interischial tuberosities and both the vertical and horizontal pelvic diameters. From this, he devised a formula to obtain a figure for the traction ratio (TR). It is as follows:

$$\text{TR} = \frac{\text{Interischial distance}}{\text{Calf's digital diameter}} \times \frac{P_1}{P_2} \times \frac{1}{\text{E}}$$

where P_1 = the party factor of 0.95 for heifers; P_2 = a correction factor of 1.05 for posterior presentation; E = a factor for breeds with muscular hypertrophy. Traction ratios greater than 2.5 are unlikely to have dystocia due to fetomaternal disproportion; between 2.3 and 2.5, traction is likely to be successful; between 2.1 and 2.3, substantial traction may be required which may not be successful; 2.1 or less, the method of treatment should be by Caesarean hysterotomy. However, a number various of the predictive equations of calf birth weight that were developed for estimation of the weight of beef calves, based upon the size of fetal extremities (Thomas 1996, Tozer et al. 2002) underestimated fetal weight of modern Holstein cows (Hiew et al. 2016). Nonetheless, the ratio between digit circumference and maternal intrapelvic area is predictive of calving difficulty (Hiew et al. 2016). The relationship is not simple, however, and is best understood in terms of the probability of a difficult calving for any fetal foot to maternal pelvis ratio (Fig. 14.32).



• **Fig. 14.32** Ordered logistic regression of the ratio of calf front foot circumference to maternal intrapelvic area as a predicted cumulative probability for the calving difficulty score. Calving difficulty score was categorised on a five point scale: 1, no assistance; 2, easy pull; 3, moderate pull; 4, hard pull; 5, mechanical extraction or Caesarean. (From Hiew et al., 2016.)

Position of the Legs. If the forelimbs are crossed over within the birth canal, it usually indicates that the elbows are being forced medially by the pressure of the pelvic wall. Likewise, if the forefeet are rotated so that the soles of the feet tend to face each other, this also indicates excessive width of the shoulder. Neither of these signs gives an absolute indication of disproportion, as they can occur with normal-sized calves.

Advancement of the Calf. Moderate traction is applied to the calf to determine whether this will advance it through the pelvis. As a rule, if no progress has been made within 10 minutes of traction, it is unlikely that the fetus can be delivered by that means. NADIS (2017) define progress in a calf in anterior presentation as follows: "reasonable traction should deliver the calf when two people pulling can extend both front legs such that the fetlock joints protrude one hand's breadth beyond the vulva within 10 minutes' traction. Such movement of the calf's forelegs represents extension of both elbow joints into the cow's pelvis". A calving jack or calf puller can substitute for the two people (see Fig. 11.7). Likewise, if it is possible to bring the head and the elbows of the two forelimbs caudal to the brim of the pelvis, it is likely that traction will be successful. Similarly, if the calf is in posterior presentation, reasonable progress is indicated by progress of the hocks beyond the vulva (Mortimer 2017). Unsuccessful traction should be abandoned after 10 minutes, as the prognosis for both

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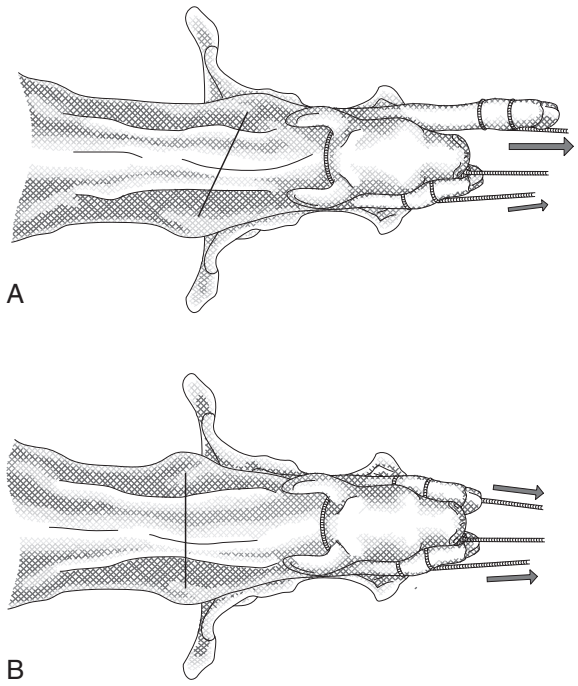

• **Fig. 14.33** Disposition of the calf: anterior presentation, dorsal position, extended posture; fetomaternal disproportion. Delivery by traction: note the use of a head snare for axial traction. (A) Traction is applied to alternate forelimbs to advance one shoulder in front of the other to reduce the diameter of the trunk. (B) If one shoulder is not advanced, the diameter of the trunk can be sufficient to prevent delivery of the fetus.

cow and calf after subsequent Caesarean hysterotomy worsens with the duration of traction.

Delivery by Traction

Anterior Presentation

The vast majority of cases of mild to moderate fetomaternal disproportion are successfully treated by the application of manual traction to the presenting feet, in many cases by farm staff. Delivery of more severe cases is expedited by first applying a head snare (see Fig. 14.2) so that an axial pull may be put on the fetus.

The animal is suitably restrained. A loop is made in the head snare, and this is carried into the vulva where part of the loop is placed in the calf's mouth and the remainder pushed up over the forehead and behind the ears. A simpler alternative, which is easier to apply and less stressful to the calf, is to push the centre point of a rope snare over the forehead and behind the ears, leaving both ends of the snare outside the vulva. A good axial pull, which also tends to depress the calf's poll ventrally, can be achieved by simultaneous traction on both ends of the snare. Each of the other snares is placed above and below the fore fetlock of the calf (see Fig. 14.1). As the head rope is held taught, traction is applied to one foot snare, with a view to advancing one shoulder through the pelvic entrance. Then the other leg is advanced in the same manner. Traction upon the head should not be excessive. In this way, one shoulder is kept in front of the other, resulting in a reduction of fetal diameter (Fig. 14.33A and B). At all times, traction should be synchronous with the expulsive efforts of the cow and, as far as practicable, the initial pulling should be upwards; once the head engages the vulva, however, the direction of traction should be obliquely downward. Birth of the head is facilitated and rupture of the perineum is less likely to occur if, as downward traction is maintained on the head snare, the obstetrician inserts both hands, 'cups' them over the occiput, and presses vigorously downwards. When the fetal head is born, all three ropes may be pulled on as the cow strains and the direction of traction should progressively approach to the near vertical. After each bout of straining and with each small advance of the fetus, the veterinarian should ascertain by further examination that delivery is proceeding satisfactorily. Once the calf's head, neck, and shoulders are delivered, the obstetrician should rotate the calf (see Figure 14.36B) at the time that further traction is applied. The aim of this is to line up the calf's hips, being the biggest diameter of its hindquarters, with the biggest diameter of the dam's pelvis, thus lowering the risk of the calf becoming 'hip-locked'. At all stages of traction it is important that the veterinarian should determine that the disposition of the calf continues to remain normal, as well as its progress through the birth canal by vaginal examination. Frequent applications of lubricant to the vagina and to the fetal occiput are indicated, and the veterinarian should be satisfied with gradual progress.

Posterior Presentation

The capacity of the fetus to survive obstructive dystocia is reduced if it is in posterior presentation; such cases therefore require prompt action. The principles of delivering a fetus in posterior presentation are similar to those of a fetus in anterior presentation: the obstetrician should first attempt to assess the degree of disparity between the fetus and the dam's birth canal. Delivery by traction should first be tried, unless there is gross disparity. Delivery of a fetus in posterior presentation can be more difficult than one in anterior presentation because of the abruptly presenting buttocks and contrary direction of the fetal hair. The tail can become reflected in a cranial direction over the calf's back, which will not only impede delivery but can also cause significant trauma to the dorsum of the cow's vagina. It should therefore be ensured that the tail is between the calf's buttocks before applying traction.

The hind feet are usually visible at the vulva. Snares are applied above and below the fetlocks, and the position of the fetal tail is checked. In delayed cases, fetal fluids should be supplemented, and copious lubrication applied to the vagina and vulva. One hindlimb is advanced in front of the other to minimise the presenting diameter of the fetal pelvis into the birth canal (Fig. 14.34A and B). Thus with one leg repelled as far as possible, the other is pulled on so as to bring its stifle over the pelvic brim. The repelled limb is then similarly managed. A simple way of assessing the likely success of traction can usually be predicted if both stifle joints can be brought into the pelvis after a moderate amount of traction.

There is a misunderstanding, particularly among some stockpersons, that calves in posterior presentation need to be pulled out very rapidly, otherwise they will die. One must remember that the calf's life will not be compromised until its umbilical cord becomes trapped against the maternal pelvis. In practical terms therefore, traction should be controlled until such time as the calf's tail-head and anus begin to emerge from the cow's vulva. Once this point is reached, delay should be avoided. If the hindquarters can be delivered, the forequarters usually follow. In cases of posterior presentation in which traction has not succeeded, the fetus must be removed by Caesarean hysterotomy or fetotomy.

Management of Delivery by Traction

As far as possible, traction should coincide with the abdominal contractions of the cow. It is not unusual for a cow to go down when heavy traction is applied; however, this is not necessarily

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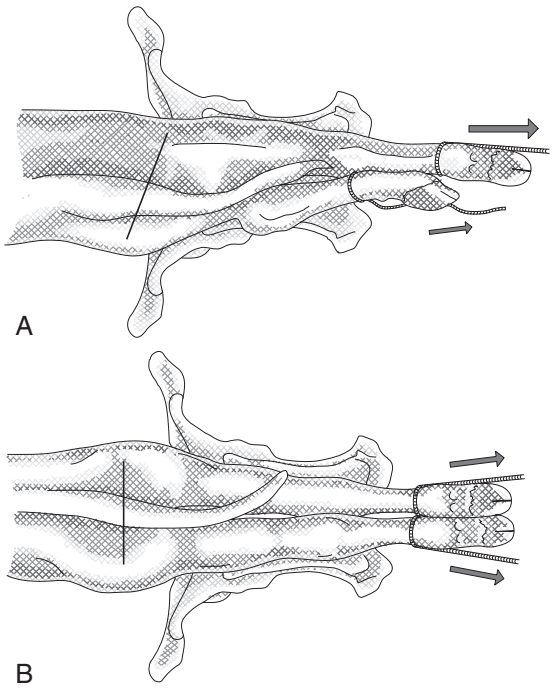

• **Fig. 14.34** Disposition of the calf: posterior presentation, dorsal position, extended posture; fetomaternal disproportion. Delivery by traction. (A) Traction is applied to alternate hind limbs to advance one hip in front of the other to reduce the diameter of the calf's pelvis. (B) If one hip is not advanced, the diameter of the pelvis can be sufficient to prevent delivery of the fetus.

• **Fig. 14.35** Fracture of the distal metacarpal epiphysis from excessive force exerted through a wrongly applied limb snare. (Courtesy Dr. Jakob Malmo.)

a disadvantage, provided that she does not fall awkwardly and injure herself. In fact, with the patient in left lateral recumbency (i.e., rumen down), traction may be applied to better advantage, particularly if manual or by means of a pulley block. However, in the case of some calving jacks, recumbency can be an inconvenience. The tractive forces exerted by calving aids and pulley blocks are much greater than those associated with natural calving or the use of manual traction by people. Despite the obvious advantages of mechanical aids to traction, there are some important disadvantages:

- The amount of excessive force that can be applied in unskilled hands;
- The fact that the pull is continuous and unrelenting, which may lead to damage of maternal soft tissues – in natural birth the calf would be advanced some way with each contraction and then go back a little before the next contraction pushes it even further; and
- The fact that the direction of pull has to be at least slightly down towards the udder. If it is horizontal or away from the udder, then the rump bar merely slips down the perineum away from the vulva when traction is applied. This means it is very difficult to apply force in the same direction as the expulsion forces of the cow. In case of anterior presentation, force should ideally be applied in a slightly upward direction until the calf's head is within the pelvis, then in a horizontal direction until the calf's head and chest have been delivered, and, finally, in a progressively more downward direction until the calf's hips have been born.

If no progress has been made after 10 minutes of judicious traction, the dystocia must be managed either through Caesarean hysterotomy or fetotomy. Fetotomy should be reserved for cases in which the calf is dead. Some oversized fetuses can be delivered by a small number of cuts (e.g., the removal of a forelimb or a forelimb together with the head and neck), but some will require delivery by means of a full fetotomy, involving five or six cuts. Note that full fetotomy is the method of choice for a calf that is emphysematous and decomposing.

Assessment of the likely success of traction to relieve dystocia due to fetomaternal disproportion is very much based on the veterinarian's experience. Although it is probably reasonable to attempt vaginal delivery in the vast majority of cases, knowing when to refrain from such an attempt or when to cease with traction and move to an alternative method of delivery is a key decision in achieving a successful outcome. Sadly, however, it is very common for the calf to have died before veterinary assistance is requested, with the result that usually it is only the cow that can be saved.

Excessive traction can cause trauma to the cow, including damage to the birth canal, tearing of the perineum (see later in this chapter), haemorrhage from uterine blood vessels, and damage to the lumbosacral plexus. Schuijt (1990) also showed that excessive force is the most important cause of vertebral and rib fractures in calves, many occurrences of which are mistaken for fetal asphyxia. Hiplock is a particular risk for fetal trauma, and underestimation of the size of the fetus carries a particular risk for vertebral fractures. Wrongly placed foot snares can result in significant damage to the legs: epiphyses can be damaged or limbs even fractured (Fig. 14.35).

Episiotomy

If the vulva is relatively small for the size of calf that is to be delivered (as is commonly the case in Holstein–Friesian heifers) and that, despite giving the vulva time to relax and stretch, it is clear that further traction on the calf risks tearing of the vulva and perineum, episiotomy should be performed. If the vulva is stretched to the point of tearing, the dorsal commissure will rip uncontrollably, usually directly towards the anus and rectum – potentially

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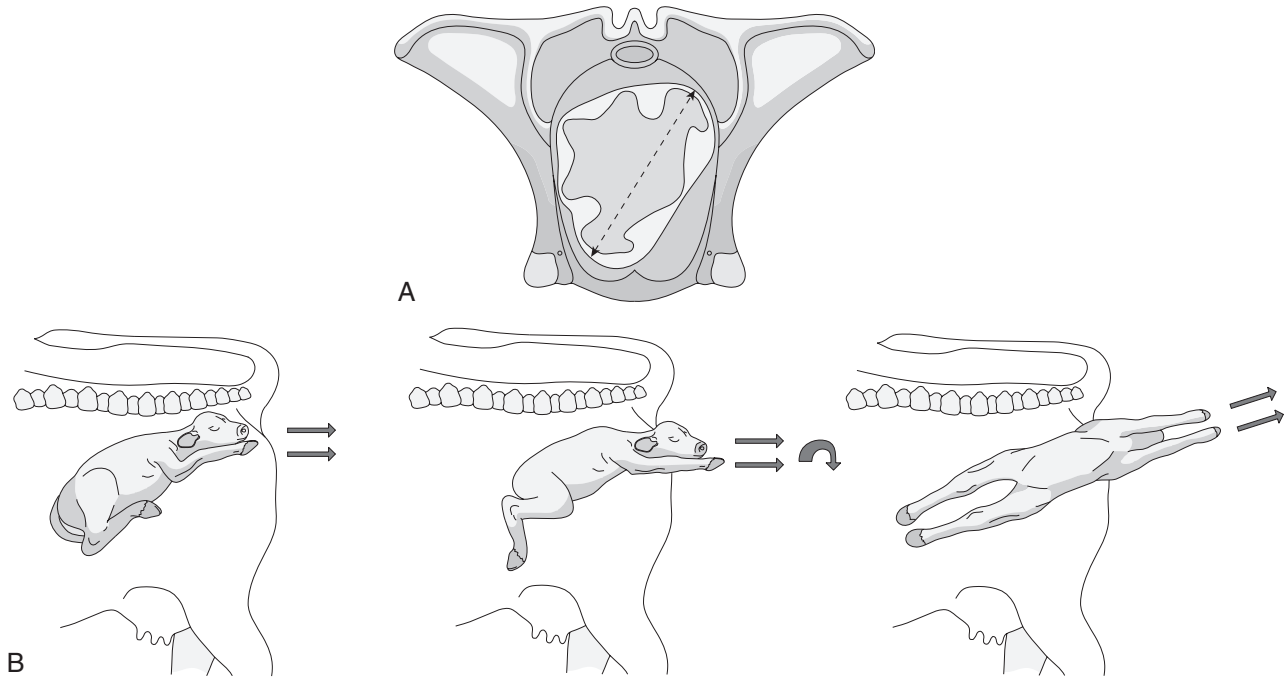

• **Fig. 14.36 (A)** Aligning the maximum diameter of the fetal hips with the maximum diameter of the maternal pelvis, to prevent the development of 'hip-lock'. (B) Rotation of the calf as a routine component of the management of fetomaternal disproportion.

resulting in a third degree perineal laceration (see Chapter 20). The risk of uncontrolled tearing can be managed by performing an episiotomy, i.e., an incision on one or both sides of the vulva (Freiermuth 1948). The upper third of the vulvar labium is incised at the junction between the mucosa and skin in the shape of a slight arc in a dorsolateral direction. Cutting directly upwards into the perineal raphe is contraindicated, as this does not reduce the risk of uncontrolled tearing through the perineum and formation of a third degree perineal laceration. The requisite depth of the vulvar incisions can be decided only by trial and error, on the basis of the minimum amount to allow delivery. By gentle traction on the fetal head so as to cause firm engagement of the occiput in the vulvar orifice, it is easy to ascertain the necessary depth of the incision. Local infiltration, rather than epidural anaesthesia, should be used so as not to interfere with the maternal expulsive efforts. However, some obstetricians believe there is little, if any, need for local anaesthesia because the vulvar lips will be quite numb at the time of maximal stretching. The episiotomy wound should be repaired by suturing immediately after delivery to re-establish normal morphology of the vulva. A deep incision may require separate repair of the skin and subcutaneous tissues, but not the mucosa layer. In most cases, however, placing deep sutures through the skin and subcutis is all that is required for repair.

Delivery of the Fetal Pelvis

Obstruction sometimes occurs as the fetal pelvis engages the dam's pelvic inlet, in either anterior or posterior presentations. This problem is variously ascribed to the greater trochanters of the femurs and the overlying muscle of the calf impinging on the shafts of the ilia of the dam ('hip-lock') or to the stifles of the calf impacting the brim of the maternal pelvis ('stifle-lock'). The umbilical cord is compressed, and the calf may try to start breathing, albeit not totally efficiently, due to compression of its abdomen in the birth canal. So long as the calf is breathing adequately, rapid extraction of the fetus is not necessary. The fetus must be disengaged from the maternal pelvis by retropulsion and rotated through an angle of 45 degrees or even 90 degrees to better align the greatest diameter of the maternal pelvis with the width of the greater trochanters (Fig. 14.36A and B). The direction of traction should now be vertically downwards until birth is completed. The nostrils of the calf are freed from amnion or mucus, and resuscitation is initiated. The genital tract of the cow or heifer is explored: first in order to ascertain that another calf is not present and second to make sure that it has not sustained significant injury.

In the case of impacted 'hip-lock' by a dead fetus when it is impossible to repel and rotate the calf to a sufficient degree to allow its delivery, the dystocia has to be managed by fetotomy. The calf is pulled as far out of the vulva as possible, its trunk is transected as close to the fetal pelvis as possible, after which the fetal hindquarters are cut in half (see Chapter 15). When this has been completed, each part of the hindquarters can then be delivered one after the other.

Similarly, in a posterior presentation, if the fetal pelvis becomes impacted in the birth canal, the calf should be repelled a little, rotated through 45 degrees, and traction again applied. This latter manipulation, which brings the greater diameter of the fetus into the largest pelvic dimension, is often successful; it may be accomplished by simply bending the protruding metatarsi and using them as levers in a rotary manner.

Fetomaternal Disproportion in the Horse

Disproportion as a cause of dystocia is uncommon in horses. Apart from being more urgent, the occasional case of relative oversize is treated along similar lines to the bovine case, with the exception that, because of the late osseous union of the fetal skull, traction should not be applied to the foal's head except to maintain its alignment with the birth canal.

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Although the length of gestation is very variable in the mare, in some cases exceeding 365 days (Rosales et al. (2017) reported an interquartile range of 343 to 355 days and a maximum of 429 days), excessively large fetuses are rare in horses. Unlike the situation in cattle, in which prolonged gestation is normally associated with fetal oversize, prolonged gestation in the mare is more usually associated with placental insufficiency and the birth of an undersized fetus. Owners can become anxious about the viability or risk of oversize of the fetus when gestation is prolonged, but neither is likely to be an issue in overdue mares (Frazer 2011). Fetomaternal disproportion, when it does occur, is usually seen in primiparous mares; such mares may need assistance in the form of traction. If it is confirmed by trial traction that the fetus is unlikely to be delivered by traction *per vaginam* then a Caesarean hysterotomy is the preferred option for management.

Fetomaternal Disproportion in the Sheep and Goat

Oversize is a common cause of dystocia in ewes carrying single lambs. Ewes of the smaller breeds are often mated to larger rams, and, although the fetal size is controlled to a large extent by the dam, bulky body features derived from the ram, such as a large head and coarseness of shoulders and buttocks, often cause difficulties. Most cases are successfully overcome by farm staff applying traction to the forelegs. More severe cases may be presented for veterinary assistance, in which they may be treated as described for the cow. When traction – using fine snares, copious lubricants, and a high standard of cleanliness – does not succeed, Caesarean hysterotomy or fetotomy may be employed. Where the fetus is dead, fetotomy is indicated. In these species the subcutaneous methods of limb removal are relatively easily carried out. Although quite practicable, the percutaneous technique, using the wire saw protected by Glattli spiral tubes, is rarely used these days.

Fetomaternal Disproportion in the Pig

Dystocia due to fetomaternal disproportion may occur in immature primigravid gilts, especially when pregnant with a small litter. In such cases it can be treated by traction using a hand, snare, or forceps. If this fails, then Caesarean hysterotomy is indicated; fetotomy is not an option.

Dystocia Due to Fetal Monsters

Monstrosities most often cause dystocia in dairy cattle, the most common example being schistosoma reflexus; next in order of frequency are: ankylosed calves, including perosomus elumbis; double monsters; dropsical fetuses, including anasarcous and hydrocephalic calves; and achondroplastic monsters (see Chapter 9). The same varieties occur, but to a lesser extent, in sheep and goats. With the notable exception of 'wryneck', monstrosities are uncommon in mares. Instances of hydrocephalus, double monsters, and perosomus elumbis occur occasionally in pigs.

Silva Filho et al. (2015: Brazil) gave an interesting account of the relative frequencies of bovine fetal monsters. They found that fetal monsters accounted for 7% of dystocias, and, of these, 35% were schistosomes and 25% were duplicate forms. Sarkar et al. (2014: India) found a slightly higher proportion (11%) of fetal monsters in cattle referred for dystocia. They reported a similar proportion of duplicate abnormalities, but only 8% schistosomes. The most common abnormality in that series was arthrogryposis (27%). However, schistosomes are not common in the overall population of cows; Knight (1996) found only 1.3% of cases of bovine dystocia were due to this cause.

With the exception of anasarcous fetuses, gross malformation is often associated with ankylosis of joints and muscular atrophy; consequently, many monsters weigh less than normal calves. This lower weight, coupled with the fact that they are sometimes associated with abortion or premature birth, means that a monster may be sufficiently small to be passed spontaneously. However, a grossly irregular development, including bending or twisting of the vertebral column and ankylosis or duplication of limbs, means that a wider than normal fetal diameter presents at the pelvic inlet, usually causing severe dystocia.

Principles of the Delivery of Monstrosities

Recognition of the exact disposition of the fetal extremities and an estimate of fetal size may be very difficult. The obstetrician must then consider whether careful traction – with due regard to lubrication and protection of the birth canal from irregularly disposed appendages – is likely to succeed. Before an attempt at vaginal delivery, the diameter of anasarcous, ascitic, and hydrocephalic fetuses may be reduced by appropriate multiple or single fetotomy incisions. If fetotomy or moderate traction does not soon succeed, a Caesarean hysterotomy must be employed. Occasionally, monstrosities present baffling problems to the obstetrician. This happens when the presenting part of the fetus is normal but the distal extremity is grossly malformed; birth proceeds normally until the malformed portion engages the pelvic inlet. The cause is not apparent and may be impossible to ascertain. Examples are provided by: perosomus elumbis in which the front half of the calf negotiates the birth canal but the ankylosed and distorted hindlimbs become impacted; a hydrocephalic fetus in posterior presentation; and cases of anterior duplication in posterior presentation. In these instances, forceful but unsuccessful traction has usually been applied before the arrival of the veterinarian. This history, together with the normal appearance of the presenting portion, should make one suspicious that an abnormality is present in the distal portion. Caesarean hysterotomy often provides the easiest solution but not necessarily the most appropriate. In many cases, a partial fetotomy, involving a few strategically carried out cuts, may resolve the dystocia.

Obstetrical Management of Schistosoma Reflexus

This most familiar bovine monstrosity requires special consideration. The features of the malformation were described in Chapter 9. Schistosoma reflexus may also occur in other ruminants, as well as in pigs, and may present viscerally or by its extremities. Some fetuses in visceral presentation are born without assistance.

With this type of dystocia, fetal viscera may be seen protruding from the vulva (Fig. 14.37A and B); if not, they are soon located within the birth canal by vaginal exploration. The fetal viscera may be mistaken for those of the mother, in which case uterine rupture may be suspected. However, it should not be difficult by careful examination to dispose of this suspicion, with the absence of a uterine tear and the continuity of the viscera with the fetus being soon established. Most schistosomes will need to be delivered by fetotomy, if in visceral presentation, or by Caesarean hysterotomy if the extremities are presented. Sometimes, however, vaginal delivery

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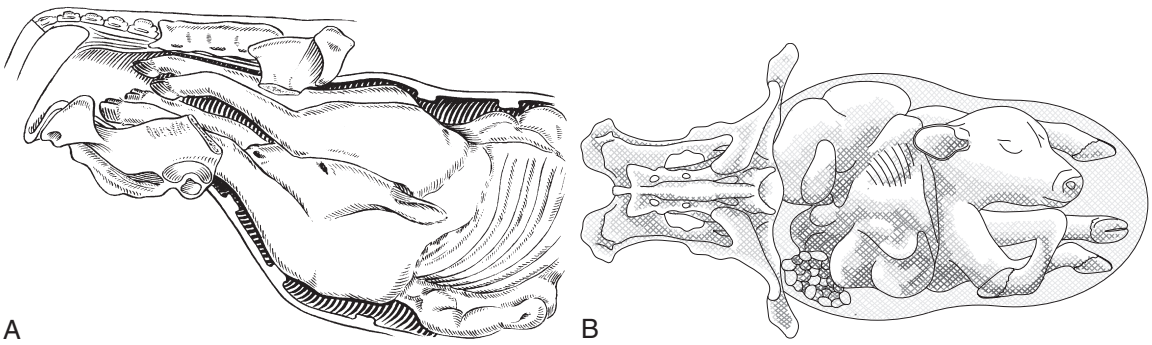

• **Fig. 14.37** Schistosoma reflexus. (A) Presented by its extremities. (B) Presented by its viscera. Fetuses presented by the viscera can often be delivered *per vaginam,* but those presented by their extremities are best delivered by Caesarean hysterotomy.

• **Fig. 14.38** (A) Friesian cow with a schistosoma reflexus calf in visceral presentation resulting in the appearance of fetal viscera at the vulva. (B) Schistosome delivered from the cow shown in (A). (Courtesy Dr. Jakob Malmo.)

without fetotomy may be possible if the fetus is small. If this is to be attempted, a Krey hook should be fastened to the presenting fetus and reasonable traction, with adequate lubrication, applied. At the same time, the veterinarian should pay particular regard to the possibility of damage by bony fetal prominences to the birth canal. In this way the expulsive efforts of the cow are gently aided, and smooth delivery may be achieved. Fig. 14.38B shows a schistosome delivered by manipulation and traction from the cow shown in Fig. 14.38A. When a schistosome presents by its extremities, the excessive fetal diameter, together with the ankylosis of joints, is likely to prevent natural or manipulative delivery *per vaginam,* and, unless the fetus is very small in relation to the maternal pelvis (as might occur in a schistosome twin to a normal calf), time should not be wasted on an attempt at vaginal delivery. Caesarean hysterotomy will be required because fetotomy is difficult in this type of presentation.

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Defects of Presentation, Position and Posture in Livestock: Delivery by Fetotomy

JOS J. VERMUNT AND TIMOTHY J. PARKINSON

By definition, fetotomy (originally referred to as embryotomy) is dissection of a fetus into two or more parts within the uterus and/or vagina. Its purpose is to reduce the size of the fetus such that delivery through the birth canal becomes possible. It is in the cow that the procedure is most frequently used, mainly because of the size of the uterus and the possibility to introduce instruments to the full extent of the fetus *in utero.* Nowadays, fetotomy is mostly carried out when the fetus is dead; however, exceptions may be when the value of the cow and her rebreeding ability and/or milk production are greater than the value of the calf. In this case the fetus should be killed first by grasping its umbilical cord, which can be reached in most cases, and severing it with a sudden pull; death follows quickly due to exsanguination. Although the fetotomy procedure can be effective in relieving dystocia in the mare, hind, ewe and doe goat, there are problems and thus limitations. For example, in the mare the vaginal wall often becomes oedematous after minimal obstetrical manipulations, resulting in a severe reduction in the lumen of the birth canal. Thus those manipulations required to perform fetotomy are likely to induce these changes quickly, making it very difficult because of reduced space. In addition, the mare is much less stoical than the cow, possibly requiring sedation or general anaesthesia. If dystocia can be relieved by one simple procedure then it may be worth considering (the techniques used are generally the same as those described later in this chapter for the cow); if not, then Caesarean hysterotomy should be considered as the method of choice. In ewes and doe goats the main difficulty with performing fetotomy is the small size of the dam. However, limited fetotomy can be done quite successfully to relieve dystocia; some shepherds are very skilled at performing a subcutaneous fetotomy (see later in this chapter) when the fetus is emphysematous and undergoing extensive putrefaction. Dystocia in hinds is frequently due to postural defects, and partial fetotomy can be carried out quite effectively in this animal species. Fetotomy is not applicable as a method of correcting dystocia in animal species that give birth to several offspring at a time. The various techniques described later in this chapter relate to the cow.

Fetotomy should not be considered as an alternative for Caesarean hysterotomy but is indicated in certain cases of dystocia. The decision guidelines based on 'diagnostic traction' (for anterior presentation, whether the fetlocks can be delivered a hands-breadth outside the vulva, or the hocks to the level of the vulva in case of posterior presentation) are a useful aid to decide whether or not fetotomy is indicated. If delivery of a dead fetus by traction is not possible without danger to the cow, the veterinarian must consider the option of Caesarean hysterotomy or a fetotomy. If the dead fetus can be delivered by the employment of limited fetotomy, such as the removal of a forelimb or a forelimb together with the head and neck, then this will certainly be the method of choice; unfortunately, having embarked on a fetotomy the obstetrician frequently finds that total dismemberment will be necessary. Because of the difficulty in assessing the extent of fetotomy required, and the knowledge that total fetotomy is a tedious and arduous task, there is an increasing tendency for veterinarians to resort to Caesarean hysterotomy in cases in which the fetus cannot be delivered by reasonable traction (see Chapter 14). The decision-making process of whether to do a Caesarean hysterotomy or a fetotomy to resolve a dystocia depends to a large extent on whether the veterinarian possesses the skill and working knowledge to perform and complete the chosen procedure equally well. It is unfortunate that, with the greater ease and increased success rates of Caesarean hysterotomy, veterinarians in many parts of the world have lost the skills required to perform fetotomy successfully.

Both Caesarean hysterotomy and fetotomy procedure have a place in veterinary practice and both should be used when appropriate. If an assisted vaginal delivery is not possible, Caesarean surgery is the method of choice if the fetus is alive and viable. Fetotomy must be given first consideration if the fetus is dead, especially when it is emphysematous and the cow is toxic. It is important for the veterinarian to be decisive, so that neither Caesarean hysterotomy nor fetotomy is used as a last resort when the cow and operator are likely to be exhausted and the birth canal traumatised. The decision of what procedure to use should be made promptly after it becomes clear that delivery by traction is not justified. A common mistake is the use of too great and too prolonged traction in the case of fetomaternal disproportion before the decision to perform either procedure is reached; about 5 minutes without any obvious progress is a reasonable period of time. In the case of faulty disposition the same considerations apply if after 15 minutes no progress has been made.

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Two basic types of fetotomy have been described in the veterinary literature, i.e., subcutaneous fetotomy and percutaneous fetotomy (Norman & Youngquist 2012). Subcutaneous fetotomy was developed in the late 18th century and is carried out with the use of knives, chisels, and hooks. Fetal parts are systematically removed while leaving the skin attached to the remainder of the fetus, which is still inside the uterus. At least one distal part of a limb is left attached to the skin, thus providing a point for attaching a calving rope or chain, and enabling traction to be applied to the rest of the fetus. In addition, the retained fetal skin perfectly protects the dam's birth canal and uterus from being damaged (Paiba 1995). Nowadays, subcutaneous fetotomy is rarely taught as a practical procedure at veterinary schools around the world; nevertheless, the procedure will be briefly outlined later in this chapter for completeness.

Since the early 20th century, percutaneous fetotomy has become the method most commonly employed, and this procedure will be described later in this chapter in detail. The technique involves the dismemberment or dissection of a fetus by cutting through skin, muscles, ligaments, and bone using braided wire (Mortimer & Toombes 1993, Frazer 1998). It is faster than subcutaneous fetotomy, but damage to the uterine wall and birth canal during cutting and manipulation of the fetus is a potential risk in all cases of percutaneous fetotomy.

In comparing the facility with which a calf may be removed by subcutaneous or percutaneous fetotomy, it must be clearly appreciated that the most troublesome part of the percutaneous method is the correct placing and retention of the wire. Given strong multifilament wire, the actual sawing process should present no difficulty. Occasionally, the two methods may be combined, e.g., the subcutaneous procedure for the forelimb(s) and percutaneous fetotomy for the head, trunk, and hindlimbs. No matter what method is used, in order to successfully perform a fetotomy, it is essential that the veterinarian has the technical knowledge and experience and uses the proper equipment plus copious amounts of obstetrical lubricant. The procedure should always be carried out under caudal epidural anaesthesia in all ruminant species (see Chapter 14) and in the mare under sedation or general anaesthesia.

Indications

In order to save the life of the cow, a fetotomy is commonly carried out under the following circumstances (Norman & Youngquist 2012, Jimenez et al. 2015):

- To correct dystocia due to fetomaternal disproportion – an oversized fetus in a normal cow or a normal size fetus in an undersized (usually juvenile) cow;
- To correct maldispositions (presentation, position, posture) of the fetus that cannot be corrected or in which correction would be an unacceptable risk to the cow;
- To correct dystocia due to the fetus and dam being in a 'hip-lock' that cannot be corrected by fetal rotation;
- To relieve dystocia caused by incomplete cervical dilatation; and
- To deliver a pathologically enlarged fetus (e.g., fetal gigantism, emphysematous calf, fetal dropsy, or anasarca) or fetal monster (e.g., schistosoma reflexus, perosomus elumbus).

Fetotomy must not be used when the birth canal is obstructed or reduced in size, as in the case of uterine torsion.

Experienced veterinarians generally agree that a combination of forced traction and fetotomy increases the risks to the cow and prolongs the calving procedure rather than shortening it. Either a partial (limited) or complete (total) fetotomy may be required to resolve dystocia. A partial fetotomy will normally suffice when dealing with a maldisposition. Usually, the offending appendage(s) can be quickly amputated, after which the fetus is delivered by traction. One should be aware that in protracted cases the uterus may be tightly contracted around the fetus and the repelling necessary to allow mutation may result in uterine rupture. Partial fetotomy is also useful in cases of a dead calf in 'hip-lock' or an abnormal fetus. If all relevant parts of the fetus are too large in relation to the cow's pelvis (oversized fetus), then a complete fetotomy will be required, usually involving four to six cuts. The size of each amputated piece should be small enough to be delivered with a minimum of traction by the obstetrician. A total fetotomy may be a difficult and physically very demanding procedure when there is extreme fetal oversize, an emphysematous calf, and the fetus is in ventral position. Furthermore, incomplete dilatation of the birth canal, a contracted uterus, or a restless cow, as well as a faulty cut, all can significantly prolong a fetotomy. However, a well-trained and experienced obstetrician should recognise a hopeless case; once a fetotomy is started, it should be completed. The whole procedure, when performed by an experienced operator, should, as a rule, take no longer than 1 to 1.5 hours. A prolonged fetotomy will only result in increased trauma to the cow's uterus and birth canal, as well as exhaustion of both the cow and operator.

Fetotomy offers a good alternative to Caesarean hysterotomy if there is enough room in the pelvic canal to accommodate and correctly place the instruments. Using the correct technique and approach, recovery time and the amount of aftercare are generally less than is needed after Caesarean hysterotomy. As mentioned before, particularly with maldispositions, it is often possible to deliver the fetus without having to do a complete fetotomy. However, most experienced obstetricians will also admit that deviation from the 'recommended approach' often results in more cuts (and consequently more time) being required to complete the procedure. Also, an incorrectly placed cut can expose sharp bone fragments that may readily lacerate the reproductive tract.

Instrumentation

A set of high-quality, correctly designed instruments (Fig. 15.1; see also Fig. 11.6) is essential to perform a percutaneous fetotomy, and these should include the following:

- Fetotome (either the Utrecht model, which essentially is a two-barrelled flat tube, or the double-tubed Swedish modification of the original Thygesen's fetotome (see Fig. 15.1)). The head of the instrument is the part that will be positioned inside the cow's uterus or vagina and in constant contact with the obstetrical wire. It should be smooth, rounded, and made of very durable tempered steel and hardened chrome to minimise problems with wire handling and cutting. Cheaper versions often develop deep grooves on the head due to the cutting action of the obstetrical wire. These grooves cause the wire to break, leading to enormous frustration on the part of the obstetrician. The Utrecht model has a handle near its butt end, enabling the instrument to be firmly gripped, and a notched plate immediately behind the handle that permits anchoring of a calving chain under tension, which greatly facilitates maintaining the correct position of the instrument. The use of a fetotome protects the cow from wire injuries and allows for cuts to be made in different planes, i.e., towards and away from the operator, as well as at right angles to the longitudinal axis of the instrument.

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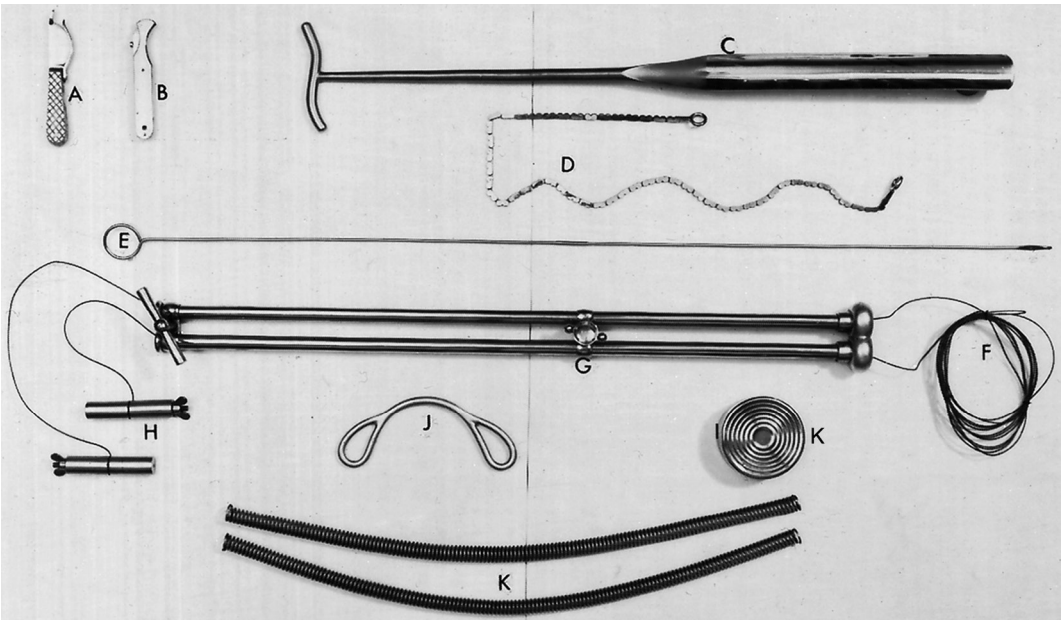

• **Fig. 15.1** Instruments for fetotomy: (A) Robert's guarded knife. (B) Unsworth's guarded knife. (C) Spatula for use in subcutaneous fetotomy. (D) Persson's chain saw, now superseded by multifilament, braided obstetrical wire. (E). Wire threader for use with (G). (F) Multifilament obstetrical wire. (G) Fetotome (Swedish modification of Thygesen's model). (H) Wire hand grips. (J) Shriever's wire introducer. (K) Gättli's spiral tubes, used to protect the genital tract from the cutting wire.

Because of the relatively large size of the modern dairy cow, it is important to use a fetotome of adequate length.

- Wire threader (see Fig. 15.1): a flexible shaft with an eye on one end for threading approximately 4 to 5 m (or four to five times the length of the fetotome) of obstetrical wire through the tube(s) of the fetotome. The Utrecht fetotome is about one meter long and may serve as a good guide for drawing out the correct length of wire. Some wire threaders have a small brush attached at the opposite end, which is very useful for clearing the barrels of lubricant, hair, and debris between cuts, and when cleaning up afterwards.
- Hand grips or wire handles – these should permit quick and secure attaching and fastening of the wire, and have a strong and comfortable grip. For ease of working, the 'split-bar'–type hand grips with a wing-shaped fastening nut are preferred.
- Krey–Schottler hook (see Fig. 11.6) – this double-jointed hook is an essential piece of equipment. When attached to a calving rope or chain, it is used to grasp onto severed portions of the fetus, particularly the vertebral column, and to apply traction to the fetus. A properly designed Krey hook should have a 'stop'; the latter is a safety feature that prevents exposure of the sharp points when the hook is closed. If this 'stop' is missing or worn, there is a real risk of a laceration occurring if the hook accidentally slips off a fetal part during traction.
- Wire introducer or guide (see Fig. 15.1) – also an essential piece of equipment. A curved introducer facilitates passage of the wire around or over a fetal part, typically the neck, a limb, or the severed hind parts. Alternatively, the wire can be attached to a calving chain or handle to assist in passing the wire around fetal parts.
- Curved finger knife (e.g., Unsworth's guarded knife) – this is only required for certain cuts. For example, it may be used to cut a 'notch' for the wire when removing a retained forelimb or hindlimb.
- Calving chains plus handles (see Fig. 11.6) – at least one chain and handle are required. The chain is used to 'anchor' the fetotome to a limb of the fetus and the handle to facilitate removal of parts of the fetus that have been cut off.
- Obstetrical or fetotomy wire – the quality varies between brands, but it is essential to select a good grade of wire. It is important that fetotomy wire is stored in a dry place (i.e., not in the obstetrics kit); it will corrode very quickly when damp or wet, causing it to become weakened. Poorly manufactured or improperly maintained (corroded) wire breaks easily.
- A pair of sharp, side-cutting pliers – these are essential for clean-cutting the obstetrical wire.
- Hand pump plus plastic tubing – this is for infusing lubricant into the uterus and around the fetus.
- Spatulas or chisels, hooks, and guarded knives, such as Robert's knife, are required for performing subcutaneous fetotomy.

Subcutaneous Fetotomy

Subcutaneous Fetotomy: Removal of a Forelimb (Anterior Longitudinal Presentation)

A foreleg may be removed by subcutaneous or percutaneous fetotomy. The simpler method, which will now be described, is subcutaneous removal, for which the essential instrument is a guarded fetotomy knife. When both forelegs are equally accessible, it is immaterial which one is removed, but the right-handed operator will find it easier to carry out fetotomy on the left forelimb of the fetus. This leg is chained – around the pastern rather than above the fetlock – and sustained traction is applied to it by an assistant. The obstetrician makes a small incision with a scalpel

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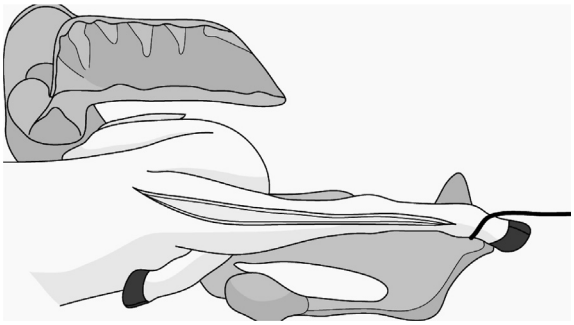
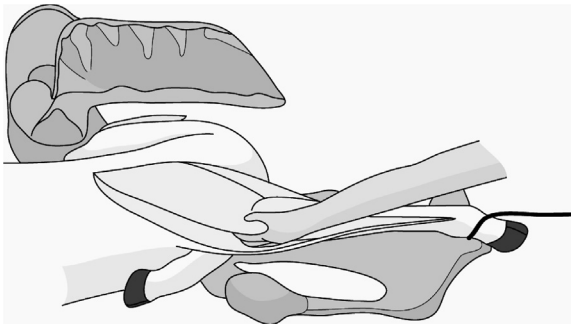


• **Fig. 15.2** Subcutaneous fetotomy, used to amputate the extended forelimb. Stage 1: the skin has been incised from the fetlock up to the scapular cartilage using a Robert's fetotomy knife.



• **Fig. 15.3** Subcutaneous fetotomy, used to amputate the extended forelimb. Stage 2: the skin surrounding the limb is dissected from the underlying tissues using fingers and hand and extending as high as possible over the scapula.

into the skin in front of the fetlock joint. Into this 'nick' the beak of a Robert's fetotomy knife is inserted, and a longitudinal incision is made up the front of the limb from the fetlock to the scapular cartilage (Fig. 15.2).

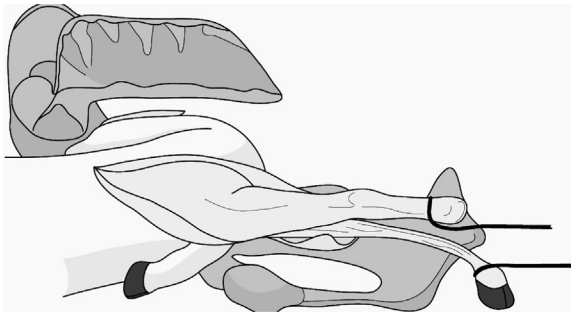
The knife is now laid aside, and the second step in the procedure is literally the 'skinning' of the limb *in situ* (Fig. 15.3). This requires strong fingers, but with application, it may be completed in about 10 minutes. (The separation of the skin from the muscles lying over the scapula completes this second step.)

The third step involves the division of the adductor muscles between the scapula and rib cage. This is achieved by reintroducing the Robert's knife, and by vigorous probing with the beak of the knife, the muscle mass is divided into several 'strings'; then each of these in turn is engaged and severed with the knife.

The fourth step (Fig. 15.4) is to disarticulate the fetlock joint so that the digits are left connected to the detached skin of the metacarpus. A chain is then attached to the metacarpus. In order to get a more secure hold, an additional half hitch may be applied above the first loop.

The chain, with a traction bar or handle attached, is then handed to an assistant. The final step in the procedure consists in avulsion of the skinned forelimb by forceful traction while the operator applies counterforce to the anterior aspect of the fetus. In this way, the remaining muscle attachments to the top of the scapula are severed and the limb comes away.

In many cases, the removal of the one forelimb gives a sufficient reduction in fetal diameter, when there is fetomaternal disproportion, to allow delivery. The principles of traction previously described are applied and, in this case the distal part and skin of the amputated limb provide a safe traction point for a chain. Should delivery not



• **Fig. 15.4** Subcutaneous fetotomy used to amputate the extended forelimb. Stage 3: after the attachments of the pectoral muscles in the axilla have been severed and the fetlock joint disarticulated, traction is applied to the 'skinned' limb. Note that the digits are still attached to the skin.

be possible after this procedure, the other foreleg must be removed in the same way, after which moderate traction is usually successful. Occasionally, after removal of one or both forelimbs – and despite partial rotation of the fetus – the hindquarters become locked at the pelvic inlet (so called 'hip-lock'; see Chapter 14). Now, the calf should be delivered as far as possible, and the protruding part of the trunk completely severed. The fetal abdomen is eviscerated, after which one of the hindlegs must be removed. There are two ways of doing this, and the one chosen will depend largely on the mobility of the retained extremity. If it is possible, the posterior part of the calf should be repelled and one of the hindlimbs brought forward with the aid of a chain; the limb is then removed by subcutaneous fetotomy (to be described later in this chapter). If it is not possible to grasp the limb and bring it forward, it must be amputated in this way: Using a direct-cutting fetotomy knife, such as Unsworth's, an incision is made over the hip joint of the leg to be removed. The muscle mass lateral to the femoral head is severed, and the upper extremity of the femur is isolated. Around this a chain is passed and the teres ligament can be ruptured by vigorous abrupt traction, and the articular head freed from the acetabulum. The chain loop is then made secure below the greater trochanter, and sustained traction is applied. This causes the leg to be drawn out from its skin; difficulty occurs over the calcaneus, but a few strokes with the fetotomy knife will free this part as well. The hind digits should be left attached to the skin and the leg disarticulated at the fetlock joint. After one of the hindlimbs is removed, the remainder of the posterior part of the fetus can be withdrawn by traction, using the Krey hook, which should be attached to the lumbar vertebral stump and the skin of the severed trunk, together with the skin and digits of the amputated limb. Amputation of both hindlimbs is rarely needed.

In cases in which 'hip-lock' occurs after partial fetotomy of the front extremity, Graham's (1979) method of causing fetal pelvic collapse could be considered as an alternative to further dismemberment of the fetus. In this method, fracture and collapse of the fetal pelvis is caused by introducing a long-handled hook through an incision in the fetal perineum. The 75 cm blunt hook engages the pelvic brim, and the fetal pelvis is fractured by abrupt backward traction. The procedure is repeated once or twice so as to ensure sufficient pelvic collapse. Traction on the unextended, lubricated breech, with the aid of Krey hooks, may then succeed.

Complete fetotomy, as described previously, is tiring and time-consuming and requires substantial skill, as well as the appropriate equipment. If the fetus is emphysematous and undergoing putrefaction the tissues may break down more readily, even with modest force, thus making the task much easier.

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Subcutaneous Fetotomy: Removal of the Hindlimb (Posterior Longitudinal Presentation)

A 'nick' is made in the skin just above the fetlock on the posterior aspect of the extended fetal leg. Into this is placed the 'beak' of a Robert's fetotomy knife, and with it, an incision is made from the fetlock up the back of the limb to the anterior gluteal region. The skin is separated from the leg, and the muscles above the hip joint, as well as the adductor muscles, are severed. The femoral head is detached from the acetabulum by introducing a traction bar underneath the Achilles tendon and forcibly rotating the limb laterally (anticlockwise in case of the left hindleg). The skin is then cut sufficiently around the fetlock joint to give scope for disarticulation, and a chain is placed over the freed end of the metatarsus. Sustained traction on the chain by one or two assistants, together with counterforce applied to the posterior aspect of the calf by the obstetrician, usually causes avulsion of the skinned limb.

After removal of the skinned leg, traction on its foot – still connected to the trunk by the skin of the leg – and on the other limb often results in delivery of the calf. If not, then the other hindlimb must be removed in a similar fashion. This will allow either complete extraction or delivery of the posterior half of the calf.

Should the forequarters become obstructed at the pelvic inlet, then further fetotomy is required as follows. As much of the calf as possible is withdrawn from the vulva and amputated. Evisceration is now carried out. The remainder is repelled and then, with an Unsworth's knife, an incision is made in the skin over the scapular cartilage, and the muscles that connect the scapula to the spine are severed. By blunt dissection the upper end of the shoulder blade is isolated, to which a Krey hook should be attached and traction applied. In this way the limb is drawn out of its skin as far as the fetlock joint, at which point it is disarticulated and removed. The digits, with skin attached, together with the Krey hook gripping the thoracic vertebral column, serve as traction points for extraction of the remainder of the calf. In rare cases the other forelimb needs to be removed before the anterior half of the fetus can be delivered.

Percutaneous Fetotomy

Some General Rules

A fetotomy, partial or complete, can be performed on both the standing or recumbent cow. Both positions have advantages and disadvantages, and sometimes there is no choice due to available, or lack thereof, facilities or the condition of the cow. From the obstetrician's point of view the standing position is far more convenient but is less safe for the cow. If the cow is sitting in sternal recumbency and unable to rise, elevation of the hindquarters will be helpful initially. As the procedure progresses, access to the fetus becomes easier with the cow in complete lateral recumbency. Alternatively, she can be placed in left lateral recumbency right from the start, with the first part of the fetus to be amputated situated uppermost. In this position the rumen will not be pushing the uterus down. In order to amputate the other extremity, the cow should be rolled over on to her right side so that part of the fetus is now uppermost. This manoeuvre may need to be repeated if a total fetotomy is to be performed.

One of the major problems during a fetotomy arises from breaking of the wire. When threading the wire, it must be handled very carefully, especially avoiding it from getting kinked. Such kinks produce weak spots at which the wire may break when sawing. Fetotomy wire, which is multifilament in design, tends to curl very easily, even after one or two cuts. Therefore it is important to examine the wire after each cut and, if necessary, to replace it if it is kinked or frayed. To lessen the chance of breaking the wire when sawing, it is advised to rotate the wire through the fetotome as each cut is made. A new length of wire should be used for each fetotomy procedure.

The procedure should be carried out as near aseptically as feasible. Several buckets of clean, warm water and disinfectant should be available: one to thoroughly clean the tail, vulva, and perineal area of the cow before starting the fetotomy; another for the instruments used during the procedure; and. finally. one or two for cleaning up afterwards.

Before Getting Started

If there are suspicions of low-grade hypocalcaemia being present, particularly in case of third parity or older cows, calcium borogluconate should be administered intravenously before starting the fetotomy procedure to reduce the chances of the cow becoming recumbent. The cow should be restrained in a calving box, head bail, or crush, ensuring that there is adequate room behind the animal, enough for manipulation of the fetotome and wire, and two to three people.

Medication

Epidural anaesthesia should be used routinely to relieve pain as well as straining (Chapter 14). In some cases, though, it may only partially stop abdominal straining. However, it does reduce the sensitivity of the birth canal and hence the pelvic reflex that will be elicited by vaginal manipulations. Tranquilisation may be indicated in case of a fractious animal, but general anaesthesia is rarely required. Administration of broad spectrum antibiotics before starting the fetotomy procedure should be given due consideration.

The use of tocolytic drugs, such as clenbuterol and isoxsuprine, is controversial. The myometrium is not responsive to these agents to any great degree if the fetus has been dead for some time – even less so when it is emphysematous. In case the uterus does respond, the chances of a fold of the relaxed uterine wall becoming trapped in the wire loop are greatly increased. If this goes unnoticed, the result could be catastrophic for the cow.

Lubrication

Lubrication is one of the main keys to success. Petroleum-based lubricants (e.g., Vaseline) are preferred to water-based products because of their tendency to stick to tissues and resist dilution by fetal fluids. However, they are more difficult to remove from arms and hands afterwards. A good alternative is a polyethylene polymer product, which should be mixed with water. However, water-based lubricants can be utilised satisfactorily, provided large volumes are frequently used. Initially, 5 to 10 litres of thick obstetrical lubricant should be infused into the uterus as deeply as possible. A wide-bore stomach tube and pump greatly facilitates the application. Ideally, the lubricant should be slightly warmed as this may improve the degree of uterine relaxation that is achieved. Regular reapplication of lubricant is required to ensure a relatively nontraumatic delivery. Insufficient use of lubricant can be, and often is, the cause of failure. Apart from facilitating the removal of the fetal segments, it also protects the delicate tissues of the cow's reproductive tract, as well as the hands and arms of the obstetrician. Skin infections on the arms of the obstetrician, usually caused by *Listeria* spp. or *Salmonella* spp., are not uncommon, especially after fetotomy of a dead fetus. Hence it is commonly advised to wear disposable

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plastic sleeves. However, these puncture or rip quite easily and also may become caught up in the wire, making the procedure much more difficult; so, many obstetricians dispense with them.

Assistance

Before starting a fetotomy, the total plan for the procedure should be properly thought through with regards to the particular case on hand. The obstetrician's task is to carefully guide the threaded fetotome through the dorsal half of the vagina and into the uterus, always making sure that the wire has not twisted or is crossed over itself. It is crucial that the wire does not come in contact with maternal tissue during the sawing process. The head of the fetotome should reach the point for final sectioning before the wire is guided forward to reach its correct position. The operator must ensure that the head of the instrument remains positioned at the intended site, at the same time paying attention to the relative position of the uterine (intercornual) septum. If one assistant is available, the obstetrician covers the head of the fetotome with one hand and maintains the position of the fetotome with the other hand. Some veterinarians like another assistant to hold the external part of the fetotome (i.e., to maintain the position of the instrument), whereas others prefer to be in complete control of the instrument themselves. With either approach, at least one assistant is required to control wire tension during its placement and to do the actual sawing. During the sawing, the operator's hand should always remain in full contact with the fetus. Sawing should not be started before a final check on the position of the fetotome has been made. Once the obstetrician has confirmed that the wire is snugly positioned around the fetus and the head of the fetotome is protected by the veterinarian's hand, sawing can begin.

The correctness, especially of the first cut, is of paramount importance. Initially, there should be slow, short and continuous sawing strokes, using moderate force. After the wire becomes firmly embedded in the tissues beneath the fetal skin, strokes of the wire can be lengthened with more pressure applied. Finally, long, continuous strokes should be made, using strong pressure. Long strokes use the maximum length of available wire, which spreads the wear and also reduces the amount of heat produced and the likelihood that the wire will break. Tension on the wire must not be relaxed during the cutting procedure nor must the sawing be stopped until it is complete. Doing so results in the wire getting trapped and damaged, causing it to break. When correctly done, the cutting action is fast, with the sectioning completed in a relatively short period of time.

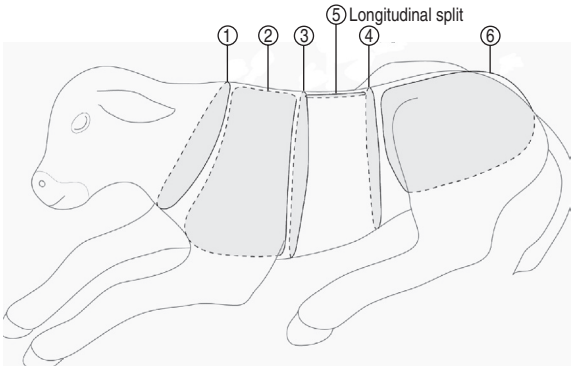
Total Fetotomy

Although the successive cuts of a total fetotomy may vary somewhat according to the individual case and the personal preference of the obstetrician, the following approach for dealing with a fetus in anterior or posterior presentation is recommended.

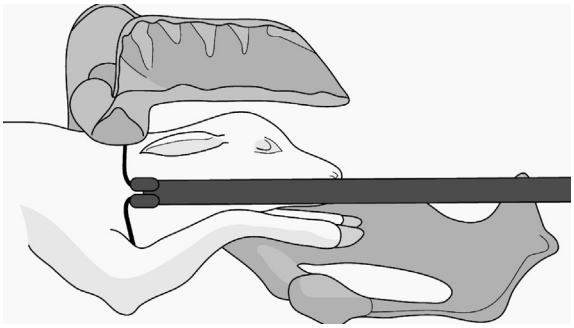
Fetus in Anterior Longitudinal Presentation (Fig. 15.5)

Cut 1: Amputation of the Head

Sometimes the fetal head needs to be removed to allow access to the forelimbs, especially if they are flexed. Both tubes of the fetotome are threaded, and the wire loop is passed over the fetal head so that it is situated behind the ears. If there is adequate room, the head of the fetotome is introduced alongside the head and positioned between the mandibles, caudal to their posterior borders. When space is limited, the instrument should be positioned on the lateral surface of the fetal head, with the head of the



• **Fig. 15.5** Drawing of a calf in anterior longitudinal presentation showing the successive cuts required for a total percutaneous fetotomy. Cut 5 is required only if the amputated thoracic/lumbar ring is too big to be extracted.



• **Fig. 15.6** Percutaneous fetotomy: positioning of the fetotome for amputating the head.

fetotome situated at or just beyond the caudal aspect of the ramus of the mandible (Fig. 15.6). If there is sufficient room, most of the fetal neck may be removed simultaneously. For this, the wire loop should be guided forwards along the neck as far as possible, and the head of the fetotome should now be placed alongside the neck, close to the scapula. After the head (with or without the neck) has been amputated, it can be extracted using a Krey hook secured into the calf's eye sockets.

Cut 2: First Forelimb

In some cases the removal of one entire forelimb (including the shoulder joint and scapula) may reduce the diameter sufficiently to permit extraction of the remainder of the fetus. A calving chain is placed around the pastern, and the leg is pulled into extension, ensuring that the distal portion of the limb protrudes from the vulva. Both tubes of the fetotome are threaded and the free end of the chain is passed through the wire loop from above. The wire loop should then be placed between the claws to briefly anchor it (Fig. 15.7). The fetotome is then advanced along the lateral aspect of the forelimb and shoulder until the head is situated above and slightly behind the dorsal aspect of the scapula. The calving chain is then anchored into the notched plate of the fetotome handle, which keeps the forelimb extended. The wire loop is removed from between the claws and guided up the medial aspect of the limb as the assistant applies slight tension on the wire handles. The wire should become seated in the axilla, and it is important to check that the wire is also situated medial to the elbow. Traction is applied to the limb once more so that, when the chain is anchored back into the notched plate, the forelimb is held in full

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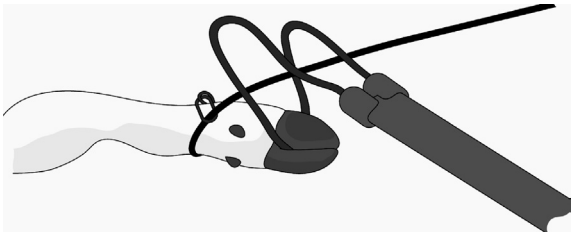
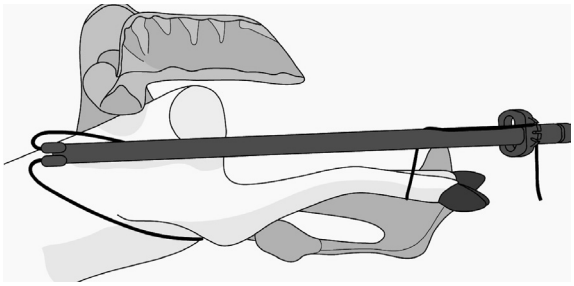


• **Fig. 15.7** Percutaneous fetotomy: temporary positioning of the fetotomy wire between the claws of a forelimb.

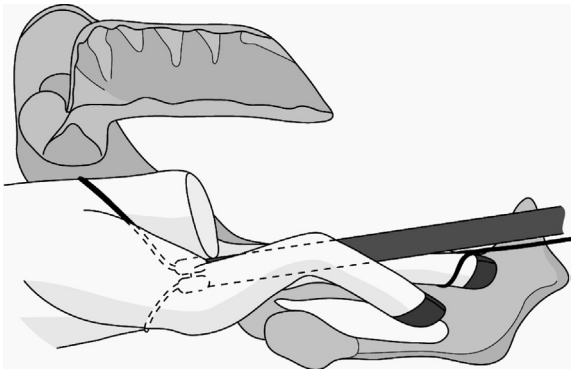


• **Fig. 15.8** Percutaneous fetotomy: amputation of a forelimb, showing the positioning of the fetotome and wire to ensure complete amputation of the extended limb.

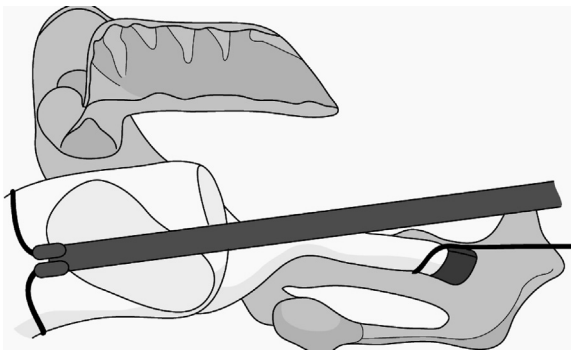
extension (Fig. 15.8). During the sawing process the obstetrician should cover the head of the fetotome with one hand. The head of the fetotome must remain dorsocaudal to the scapula for an acute-angled cut; this will ensure that the wire cuts through the muscle attachments so that the entire scapula will be removed. If the fetotome is allowed to move backwards by the assistant's sawing action, the cut is likely to be made through the distal scapula or, even worse, the shoulder joint or proximal humerus. In the latter case the diameter of the fetus is not reduced to any extent (because the shoulder joint is not removed), and there is a very sharp piece of bone left, still firmly attached to the remainder of the fetus. Furthermore, a suitable traction point (for anchoring a calving chain) no longer exists on that side. If the cut has been made through the scapula, the retained piece of bone must be detached manually by severing the muscle attachments one by one, which can be quite difficult, especially in the case of a fresh fetus.

An alternative technique is to loop the wire around the neck stump and forelimb and to push it backward along the outside of the elbow and scapula so it will lie behind the caudal angle of the scapula, where a deep incision has been made with a finger knife to accommodate the wire. The head of the fetotome is brought up to the base of the neck on the side opposite to the foreleg being removed (Fig. 15.9). With the wire loop correctly placed, the cut is easily completed. After this cut, an attempt may be made to deliver the remainder of the calf by traction. A chain is placed on the attached limb, and with the aid of the Krey hook, another point of traction is available on the neck stump (i.e., the exposed lower cervical vertebral column). If an oversized fetus still cannot be delivered after the first two cuts (i.e., head/neck and one complete forelimb) have been made, another three to five cuts may be necessary to completely dismember the remainder of the fetus.

Sometimes, it is possible to remove the head, neck, and one forelimb all in one piece. To do this, the fetotome is introduced and positioned as described previously for the forelimb, but the wire loop is now guided along the opposite side of the head and neck until it is seated near the thoracic inlet. If done correctly, this



• **Fig. 15.9** Percutaneous fetotomy: an alternative approach to amputate the left forelimb and neck after amputation of the head.



• **Fig. 15.10** Percutaneous fetotomy: transverse division through the trunk after amputation of the head/neck and right forelimb.

cut then runs obliquely across the fetal neck and thorax, creating an opening in the thorax through which complete evisceration of both thoracic and abdominal contents is carried out. Now, the fetus may be partially or completely extracted, depending on its size, by using the remaining forelimb and the vertebral stump as traction point.

Cut 3: Second Forelimb

This may be removed in a similar manner to that described previously for the first forelimb. Often, it is possible to have the wire loop situated on the opposite side of the neck stump or thoracic inlet. For this cut in particular, it is important that the fetotome remains in direct contact with the skin of the fetus and is not mistakenly advanced into the nonpregnant horn before the sawing process is started. If this happens, part of the uterine septum will be amputated, with catastrophic results for the cow.

If there is adequate room, all or at least a large part of the thorax may be incorporated in this cut. The wire loop is guided around the thorax and positioned as far back as possible (Fig. 15.10). It is important that the sawing is continued until all ribs and vertebral column are completely severed. If the sawing action is interrupted, there is a high probability of the wire getting stuck in bone tissue. Trying to free the wire will usually cause it to break. Once this transverse cut has been completed, the detached segment of fetus is carefully withdrawn from the birth canal, using the Krey hook attached to the vertebral stump as a second traction point. If carried out correctly, the thorax will now be cut to such a degree that all thoracic and abdominal organs can be removed by hand; the evisceration will substantially reduce the overall size of the remainder of the calf.

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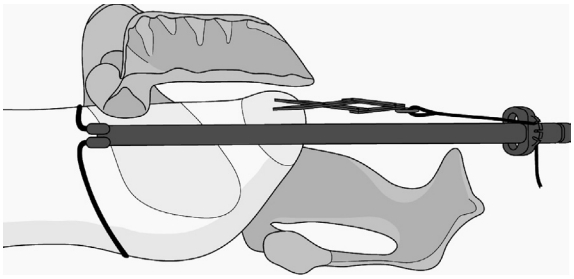


• **Fig. 15.11** Percutaneous fetotomy: division of the thorax with a single cut showing the position of the obstetrical wire behind the sternum.

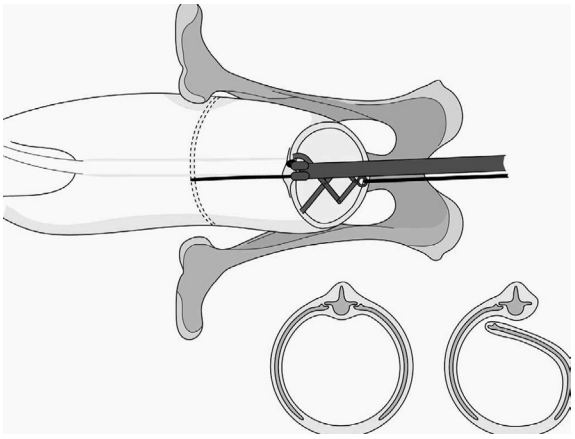
Cut 4: Thorax

The Krey hook is attached to the severed vertebral column, and the chain is passed through the wire loop of the prethreaded fetotome from above. The wire loop should be held just outside the vulva, as the fetotome is advanced over, or along, the dorsal aspect of the fetus to just behind the last ribs (if possible). The Krey hook is pulled tight, and the chain is anchored into the notch plate. The wire loop is then guided around the thorax, using a 'sweeping' motion, to end up just behind the sternum (Fig. 15.11). Before sawing, the operator should check the position of the wire to ensure that the fetotome was not rotated when being positioned and that the wire did not become twisted (best done by running the fingertips along the head of the fetotome and checking the wire inlets). During the sawing procedure the obstetrician should hold both the Krey hook and the shaft of the fetotome with one hand, while holding the handle of the fetotome with the other. Some sideways movement of the head of the fetotome should be anticipated during the sawing process, but this may be minimised by also grasping some fetal skin with the hand that holds the Krey hook and the fetotome inside the cow. Initially, short sawing strokes should be used until the wire becomes properly embedded. Extraction of the amputated chest (or part thereof) is generally fairly easily accomplished by controlled traction on the Krey hook.

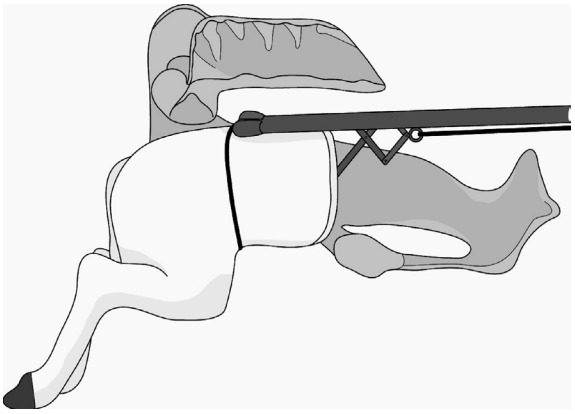
If it turns out that this portion of the thorax is still too big to extract safely, then another longitudinal cut is required to reduce its diameter by severing the ribs. The wire is removed from one of the fetotome tubes and attached to an introducer. When attaching the wire, it is important to leave a relatively long tail on the loose end of the knot to prevent it from acting as a cutting device. With the fetotome held outside the cow, the introducer is advanced over the dorsal aspect of the thorax and pushed down over the edge of the amputation site. A hand is inserted into the thoracic cavity and pushed through the diaphragm to retrieve the introducer with the wire attached. The wire is then looped back through the thorax and the fetotome rethreaded. The head of the fetotome is positioned adjacent to the Krey hook, which is anchored to the vertebral stump. It is important that the cut is made through the ribs near their vertebral attachments (Fig. 15.12). Now, this part of the thorax can be collapsed (more or less folded into a compact mass) for easy delivery.

Cut 5: Lumbar Area

If the fetus is very big, it may be physically impossible to guide the wire around the remainder of the fetus; thus it will be necessary to amputate the lumbar area first (Fig. 15.13), before bisecting the hindquarters. The approach is the same as described for the thorax section.



• **Fig. 15.12** Percutaneous fetotomy: longitudinal cut required to reduce the diameter of the thorax.



• **Fig. 15.13** Percutaneous fetotomy: positioning of the fetotome and wire to amputate the lumbar area.

Cut 6: Hindquarters

The final cut involves a longitudinal division of the hindquarters. Only one tube of the fetotome is threaded. The Krey hook should be attached to the vertebral stump and the hindquarters pulled towards the cow's pelvic inlet. The wire introducer, with the wire attached, is advanced over the dorsal aspect of the sacrum and the tailhead and pushed down behind the perineum. It is retrieved from below by passing a hand underneath the calf and between the hindlimbs. It is important to confirm that the wire is indeed retrieved from between the hindlimbs (there should be a stifle joint on either side of the retrieving hand). The wire is then pulled out and the fetotome rethreaded. Ideally, the hindquarters should be divided into equal (more or less) parts. For this, the wire should be situated on one side of the tail while the head of the fetotome is placed against the opposite side of the vertebral column, adjacent to the Krey hook (Fig. 15.14). This ensures that the cut through the fetal pelvis is made in only a slightly diagonal manner. Now, each half can be withdrawn in turn, using the Krey hook.

In some cases of dystocia, either due to fetomaternal disproportion or as a result of inadequate rotation of the calf during delivery by traction, the forequarters of a fetus in anterior presentation are delivered and then the situation of a 'hip-lock' develops (see Chapter 14). A partial fetotomy can be used to quickly relieve the dystocia. The forepart of the calf is amputated by a transverse cut through the lumbar area. The fetus is eviscerated, after which the introducer, with the wire attached, is passed over the dorsal aspect

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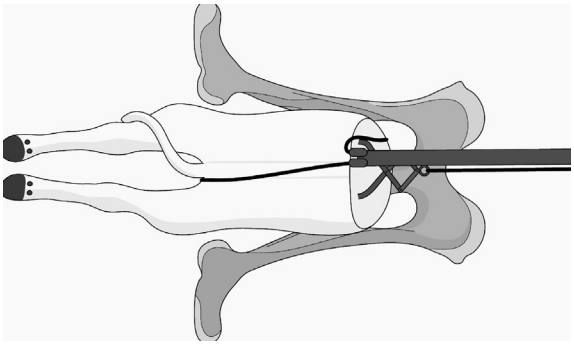
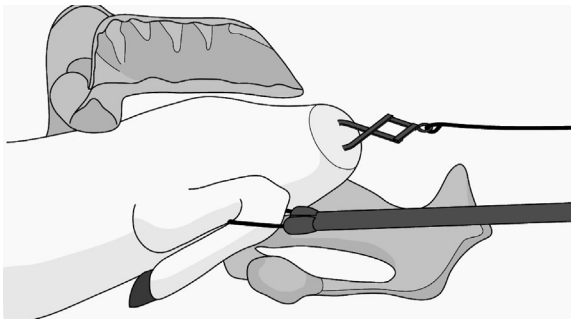


• **Fig. 15.14** Percutaneous fetotomy: positioning of the fetotome and wire to bisect the hindquarters.



• **Fig. 15.15** Percutaneous fetotomy: carpal flexion with the fetotomy cut made through the distal part of the carpal joint.

of the hindquarters and retrieved ventrally. The cut is completed and the divided hindquarters delivered as described previously.

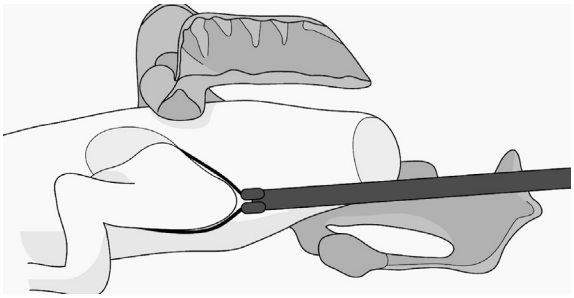
Correction of Fetal Malpostures

Carpal Flexion

Dystocia due to uni- or bilateral carpal flexion can be difficult to correct manually, especially if the dystocia has been prolonged and the uterus is contracted. It may not be possible or safe to repel the fetus and correct the faulty disposition without the risk of rupturing the uterus. In these cases the dystocia can be relieved by partial fetotomy. The head and neck should be amputated first, followed by a fetotomy cut that should be made through the distal part of the carpal joint. One tube of the fetotome is threaded, and the introducer, with the wire attached, is passed around the flexed carpal joint. After rethreading the fetotome, the head of the instrument should be positioned against the distal row of carpal bones (Fig. 15.15). Now, the distal forelimb is amputated and removed. If done correctly, there will be no sharp bone fragments, and a calving chain can be anchored against the tuberosities of the distal radius, providing an excellent traction point for delivery of the remainder of the fetus by traction. If the cut is made too high, i.e., through the distal radius, a sharp piece of bone will be the result, and there will be no traction point for the chain. The obstetrician will also need to deal with a sharp piece of bone if the cut is made too low, i.e., below the joint.

Unilateral or Bilateral Shoulder Flexion: Percutaneous Removal of a Retained Forelimb(s)

In many cases, extravulval decapitation needs to be performed first, to provide more space for subsequent procedures. A disadvantage is that this removes a point of attachment for a calving



• **Fig. 15.16** Percutaneous fetotomy: position of the fetotome and wire for amputation of the forelimb in case of shoulder flexion.

chain to apply traction; therefore a Krey hook will be required as a substitute. A finger knife is used to make an incision through the skin just medial to the dorsal aspect of the scapula and then down into the connective tissue between the scapula and thorax. In the first instance, only one tube of the fetotome is threaded, and the wire is attached to the introducer, which is then passed over the scapula and down between the thorax and the reflected forelimb. The wire is retrieved from below, and the fetotome is rethreaded. The instrument is advanced along the neck until it becomes positioned at the medial aspect of the shoulder and then held in that position. The free hand pushes the wire loop into the previously made incision (Fig. 15.16). Tension is applied to the wire to ensure that the loop is situated medial to the body of the scapula. During the sawing process the head of the fetotome must be maintained medial to the shoulder joint. Alternatively, the head of the fetotome can be positioned dorsal to the posterior angle of the scapula. However, considerable force is now required to maintain the instrument in this position when the muscles, which attach the limb to the trunk and the skin at the base of the neck, are cut. The amputated forelimb is withdrawn, using the Krey hook.

In case of bilateral shoulder flexion, an attempt should again be made to extend the other retained forelimb. If this is possible, traction on both the limb and the neck, using the Krey hook on the latter, should result in delivery. Alternatively, the fetus may be withdrawn without extending the other foreleg. If both these attempts fail, then the other, now mutated forelimb must also be removed. The subsequent procedure is the same as that described previously for fetomaternal disproportion of the second forelimb.

Lateral Deviation (Flexion) of the Head

In very obstinate neglected cases of dystocia due to lateral deviation of the head with the fetus being dead and in the occasional congenital rigid curvature of the neck called 'wryneck', correction is impossible and decapitation is required. 'Wryneck' is a relatively common disorder of the fetal foal, and this is a useful method of correcting this type of dystocia in the mare.

One tube of the fetotome is threaded, and the end of the wire, attached to the introducer, is passed in, over, and around the fetal neck. It is best achieved by first pushing the introducer over the neck, then between the neck and thorax, before retrieving it from beneath the neck. Once the fetotome has been rethreaded, it is advanced until the head of the fetotome is positioned adjacent to the thoracic inlet (Fig. 15.17). Tension should now be applied to the wire so the operator can check that the wire loop is seated as close to the thorax as possible. Once the cut is made, the severed neck can be grasped, either by hand or with the use of the Krey hook, and the neck plus attached head can be removed. Alternatively, the detached head and neck can be repelled into the uterus to be

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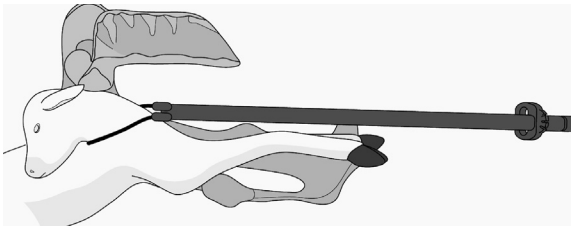
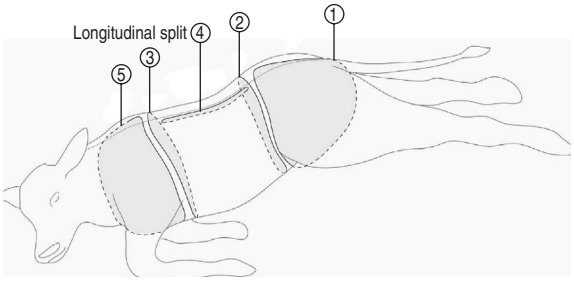
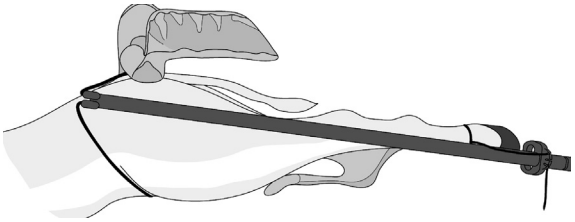


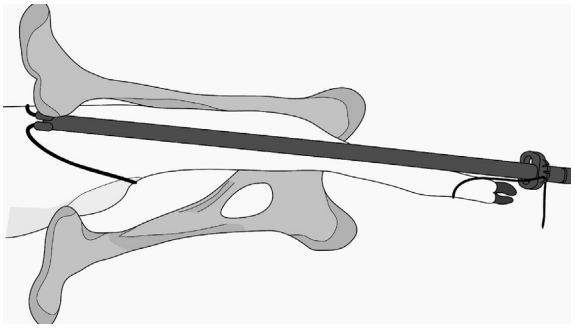
• **Fig. 15.17** Percutaneous fetotomy: position of the fetotome to correct dystocia due to lateral deviation (flexion) of the neck by amputation of the head and neck.



• **Fig. 15.18** Drawing of a calf in posterior longitudinal presentation showing the successive cuts required for a total percutaneous fetotomy. Cut 4 is required only if the amputated thoracic/lumbar ring is too big to be extracted.



• **Fig. 15.19** Percutaneous fetotomy: amputation of a hindlimb, showing the positioning of the fetotome and wire to ensure complete amputation of the extended limb.



• **Fig. 15.20** Percutaneous fetotomy: position of the fetotome to amputate the second hindlimb.

retrieved later, when the fetotomy is completed or the remainder of the fetus is delivered. In some cases of lateral deviation of the head, access to the flexed neck can be facilitated by first amputating the forelimb on the side opposite that to which the head is displaced (as described previously for fetomaternal disproportion of the first forelimb).

Downward Displacement of the Head (Ventral Flexion of the Neck)

Downward deviation of the head is rare in cattle; still, this postural defect can prove very difficult to correct by means of manipulation, and amputation of the head is indicated in obstinate cases. In this situation the introduction and correct placing of the wire between the markedly flexed neck and chest can be difficult to achieve. With the help of the introducer, the wire should be passed underneath the flexed neck. The head of the rethreaded fetotome is positioned on top of the neck between the shoulder blades. When the cut has been made, the fetus should be repelled into the uterus before the amputated head and neck can be retrieved and removed.

Fetus in Posterior Longitudinal Presentation (Fig. 15.18)

Cut 1: First Hindlimb

If the hindlimbs are extended, a similar procedure to that described for the extended forelimb can be used. Both tubes of the fetotome should be threaded. A calving chain is attached to the pastern of the hindlimb to be amputated and passed from above through the wire loop, which is then temporarily placed between the claws. Now, the fetotome is advanced along the lateral aspect of the hindlimb until the head of the instrument is situated near the greater trochanter of the femur. After the chain is anchored into the notch plate of the fetotome, the wire loop is removed from the interdigital space and guided up the medial aspect of the limb. The wire should be positioned between the tail and the ischiatic tuberosity opposite to the leg to be amputated. It is important to check that the wire is also situated medial to the stifle joint. Traction should then be placed on the chain to fully extend the entire leg. The fetotome is advanced further until the head is positioned just beyond the greater trochanter near the lumbar vertebrae (Fig. 15.19). The chain is anchored back into the notch plate and the limb amputated. If properly carried out, this cut will be diagonal across the pelvis, removing the entire hindlimb, including the hip joint. Now, the width of the fetus is reduced such that it may be possible to extract the remainder of the calf. A chain is placed on the attached limb and, with the aid of the Krey hook, another point of traction is available on the perineal area of the severed pelvis. If the fetotome inadvertently moves backwards during the sawing process, the cut will be made through the proximal femur; as a consequence the width of the fetal hindquarters is not reduced, and there is also a sharp piece of bone to deal with.

An alternative approach is to place the wire loop over one foot and pass it up the limb laterally, so that it lies anterior to the tuber coxa in which a deep cut in the skin and underlying tissues, previously made with a finger knife, helps to retain it. The head of the fetotome is positioned next to the anus, on the opposite side of the leg to be amputated. It is important to make sure that the tail and tailhead of the calf are included in the wire loop. If not, the wire may slip down the limb during the sawing process and the cut will be made through the distal third of the femur.

Cut 2: Second Hindlimb

If delivery is not possible after removing one hindlimb, the second limb can be removed in a similar manner (Fig. 15.20). It is often possible to pass the wire loop past the fetal pelvis so it is situated in the lumbar area, just caudal to the last rib (Fig. 15.21). In this situation the fetotome should be positioned further forward on the fetus. This cut allows delivery of the remaining hindlimb along with the entire fetal pelvis. Alternatively, the prethreaded fetotome can be advanced over the dorsal aspect of the pelvis and lumbar spine, and the wire loop guided up ventrally. Now, the resulting transverse cut allows extraction of the hindlimb and the remainder

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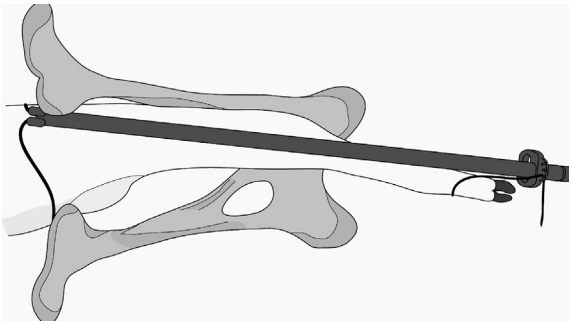
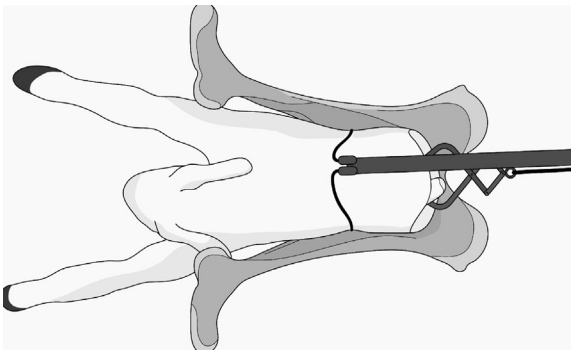
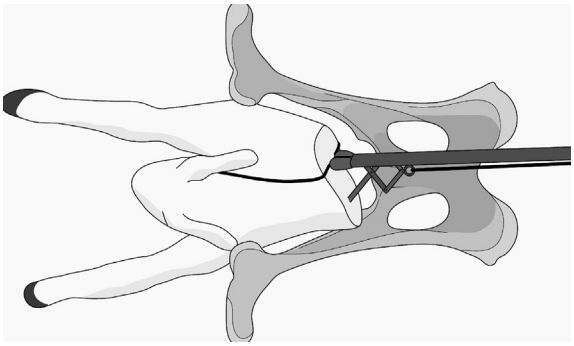


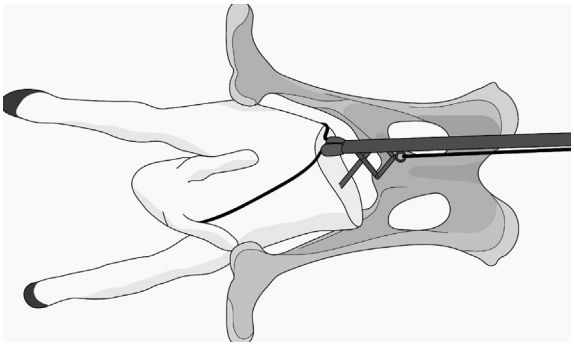
• **Fig. 15.21** Percutaneous fetotomy: position of the fetotome and wire to amputate the calf's pelvis and second hindlimb.



• **Fig. 15.22** Percutaneous fetotomy: position of the fetotome and wire to bisect the thorax.



• **Fig. 15.23** Percutaneous fetotomy: position of the fetotome and wire to amputate one complete forelimb.



• **Fig. 15.24** Percutaneous fetotomy: position of the fetotome and wire to diagonally divide the forequarters.

of the pelvis. If both hindlimbs have been removed, the Krey hook should be attached to the fetal pelvis and the wire loop positioned transversely around the lumbar area of the fetus. Once the pelvis has been removed, the remainder of the fetus is drawn towards the cow's pelvic inlet and eviscerated before the next cut is made.

Cut 3: Thorax

If the remainder of the calf cannot be extracted, then its trunk must be bisected, the transverse division being made as far forward as possible. Both tubes of the fetotome are threaded, and the fetus is pulled towards the cow's pelvic inlet, using the Krey hook which is attached to the vertebral stump. The chain is passed from above through the wire loop, which is held outside the cow's vulva. Now, the fetotome is advanced over the dorsolateral aspect of the thorax, so that the head of the instrument becomes situated just caudal to, or between, the calf's scapulae (Fig. 15.22). The wire loop is then guided around the thorax (using a 'sweeping' motion), ensuring that, ventrally, it becomes positioned just cranial to the sternum. The fetotome is held in position by the same hand that holds the Krey hook. When the diameter of this section of the fetus is still too large to allow delivery, then the rib cage should be cut longitudinally and collapsed, as described for fetotomy with the fetus in anterior longitudinal presentation.

Cut 4: The Forequarters

If it is still not possible to extract the anterior portion of the fetus, using the Krey hook attached to the severed vertebral stump, then one or if necessary both forelimbs need to be amputated. Only one tube of the fetotome is threaded, and the wire, with the aid of the introducer, is passed forwards between the neck and the limb, where it is retrieved by reaching underneath the calf. The wire, positioned between the elbow joint and the chest, is withdrawn and the fetotome rethreaded. The head of the instrument should be positioned against the severed end of the vertebral column, next to the Krey hook (Fig. 15.23). The severed limb(s) and the remainder of the fetus may be brought out one by one, using the Krey hook.

An alternative method is to diagonally divide the forequarters, in which case usually only one cut is required. Again, only one tube of the fetotome is threaded and the Krey hook attached to the vertebral stump. The wire loop is completed as described previously, but now the head of the fetotome is either placed on the opposite side of the Krey hook or against the lateral aspect of the opposite thoracic wall (Fig. 15.24). In the latter case some blunt dissection is required (i.e., creating a little pouch) before the head of the fetotome can be positioned firmly between the scapula and the thorax. When starting the cut, short strokes with the wire should be made. Once the wire has become fully embedded, the head of the fetotome is moved to the lateral surface of the scapula and the cut continued using long strokes. If this oblique cut is carried out correctly, the larger portion will be one forelimb and the main part of the thorax, whereas the other portion comprises the head and neck, the other forelimb, and the remainder of the thorax. These portions can now be withdrawn one by one, paying attention to any sharp bone fragments.

Correction of Fetal Malpostures

Hock Flexion

In cases of uni- or bilateral hock flexion, amputation of the limb(s) virtually eliminates the risk of uterine rupture that may accompany attempts to repel the fetus and correct the malposture, especially

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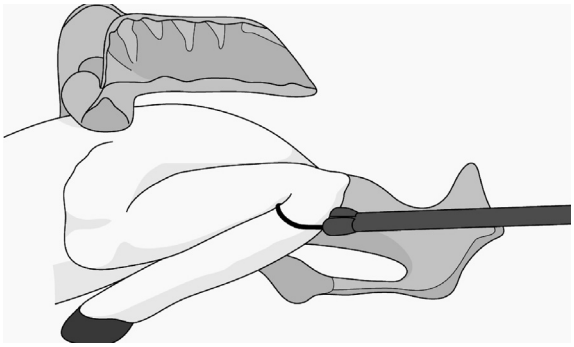
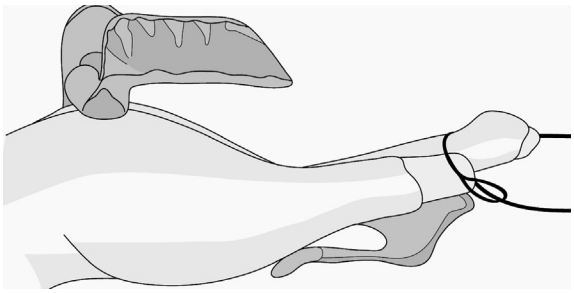


• **Fig. 15.25** Percutaneous fetotomy: hock flexion with the fetotomy cut made through the distal part of the tarsal joint.



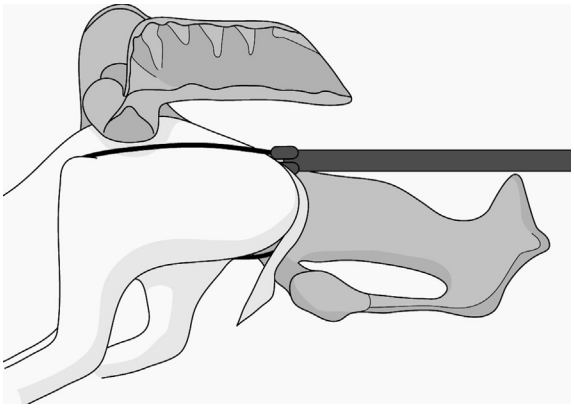
• **Fig. 15.26** Percutaneous fetotomy: showing how amputation at the level of the hock should be done to ensure that there is a still a traction point left (right- vs left hindlimb), but without the presence of sharp bone fragments.

in protracted dystocia cases. A similar approach to that described for carpal flexion should be used (Fig. 15.25). The main aim is to retain a point of traction for the calving chain and not to have sharp bone fragments (Fig. 15.26).

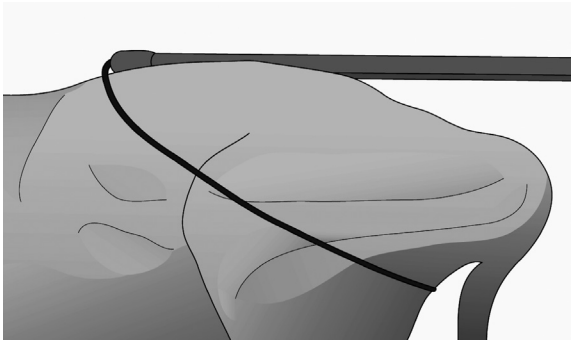
Hip Flexion

One tube of the fetotome is threaded and the free end of the wire, attached to an introducer, is passed over the dorsal aspect of the fetus and directed down between the body wall and the stifle of the most accessible retained hindlimb. Now a hand is passed in underneath the calf and the introducer retrieved ventrally, thus completing the loop. After rethreading the fetotome, the instrument is passed along the vagina, and the head of the fetotome is placed against the perineum of the calf. At this stage a crucial step in the procedure is to include both the fetal tail and tailhead in the loop of the wire and, when sawing takes place, hold the head of the fetotome firmly against the ischiatic tuberosity opposite to the leg being amputated (Fig. 15.27). In this way the femur will be sectioned through its articular head. After removal of the severed limb, the hip flexion of the other retained hindlimb can often be corrected because more room has now been created for mutation. If this hip flexion cannot be resolved safely, the retained hindlimb should be similarly amputated.

In cases in which the calf's posterior end is jammed tight into the cow's pelvis, it may be difficult, if not impossible, to repel the calf, and an alternative approach may be chosen if the calf is relatively small. The prethreaded fetotome is advanced along the dorsal aspect of the calf until the head of the instrument is situated above the last ribs, and the wire loop is placed around the back of the calf, approximately 2 to 3 cm below the calf's ischiatic tuberosities (Fig. 15.28). The resulting cut severs both hindlimbs



• **Fig. 15.27** Percutaneous fetotomy: position of the fetotome and wire for amputating the hindlimb in case of hip flexion posture.



• **Fig. 15.28** Percutaneous fetotomy: position of the fetotome and wire when using an alternative approach to manage a case of bilateral hip flexion.

through the proximal femur and continues through the abdomen, finishing at the thoracolumbar region. The amputated hindlimbs are removed one by one, followed by the pelvis, the abdominal viscera, and finally the anterior part of the fetus, using the Krey hook.

Fetal Monsters

General Principles

Fetal monsters are occasionally encountered as causes of dystocia in cattle. The diversity of abnormal conformations may test the resourcefulness of many obstetricians. Because of the worthless nature of fetal monsters, fetotomy, whether partial or complete, to achieve vaginal delivery should be given primary consideration. When the fetus is alive, it must be killed first by manually severing the umbilicus. If it is possible to reduce the fetal diameter, by making a small number of simple cuts to enable a vaginal delivery, then percutaneous fetotomy should be attempted. Thus for ankylosed fetuses, including 'wryneck' and perosomus elumbus, for cases of anterior duplication, and for schistosomes presented viscerally, fetotomy is indicated. The hydrocephalic fetus, whose cranial bones are too hard to be reduced by puncture, must have its head's dome sawn off using obstetrical wire. If the entire head is amputated, then the difficulty of withdrawing the grossly enlarged head still remains. In cases of fetal ascites ('water belly'), typically only the anterior half (head, neck, and forelimbs) of the fetus can be delivered, with the grossly distended abdomen preventing further extraction (see Chapter 9). Now, a finger knife is used to incise the fetal abdomen at several sites, enlarging the incisions

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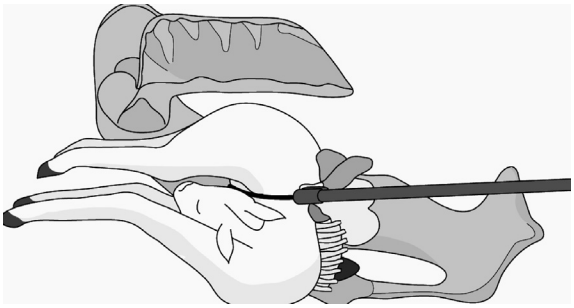
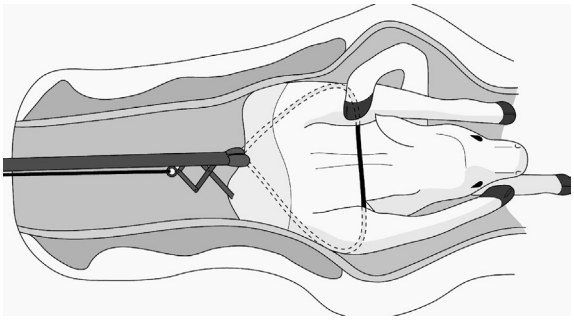
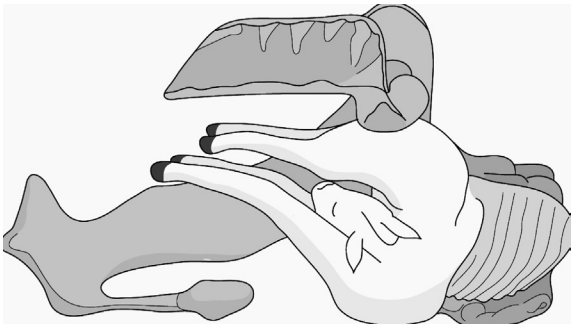


• **Fig. 15.29** Percutaneous fetotomy: position of the fetotome and wire to bisect the fetal trunk of a viscerally presented schistosoma reflexus calf after removal of the viscera.



• **Fig. 15.30** Percutaneous fetotomy: alternative approach for bisecting a viscerally presented schistosoma reflexus calf.



• **Fig. 15.31** A schistosoma reflexus calf presenting by its extremities.

by hand. During controlled extraction of the fetus, the excess abdominal fluid will escape into the uterus. Alternatively, a partial fetotomy, similar to that described for 'hip-lock', can be carried out. However, if the abdominal cavity is not opened first, it may be very difficult, if not impossible, to retrieve the introducer from underneath the hugely distended abdomen. Perforation of the diaphragm will release most of the abdominal fluid, and it may now be possible to pass the wire around the hindquarters. Cases of fetal anasarca (see Chapter 9) cause dystocia because of the large increase in fetal volume associated with the accumulation of excess fluid in subcutaneous tissues, particularly of the head and hindlimbs. Delivery of severely affected fetuses usually requires partial fetotomy or multiple incisions of the subcutaneous tissues.

When it is obvious that several fetotomy sections will be required because of excessive fetal size (as in anasarca and extensive duplication) or a very irregular presentation, the obstetrician should resort to a Caesarean hysterotomy. This will be less demanding for the operator and, in general, better for the immediate health and future breeding potential of the cow.

The same principles and considerations apply to dealing with monstrosities in sheep, goats, and deer.

Percutaneous Fetotomy in the Obstetrical Management of Schistosoma Reflexus

The more common presentation is for the fetus to be presented with its viscera exposed. First, a thorough examination should be carried out to ensure that rupture of the uterus has not resulted in prolapse of maternal organs. After killing and eviscerating the calf, usually only one to three cuts will be required on these relatively small fetuses. Before embarking on the fetotomy procedure itself, it is wise spending some time making oneself familiar with the presented case, as correct placement of the wire is the most difficult part of the entire procedure. The rigidity of the curved spine and ankylosed limbs prevents repelling of any fetal part, and inappropriate cuts will leave many sharp bone fragments that can easily traumatise the birth canal. The vertebral angulation should be clearly identified, as this is the site of the first fetotomy cut in order to bisect the fetal trunk (Fig. 15.29). One tube of the fetotome should be threaded and the wire, attached to an introducer, and carried inside in a similar manner to that described for amputating a flexed carpus or tarsus. If necessary, a finger knife can be used to make incisions in the large skin folds, thus facilitating the ventral retrieval of the introducer. The Krey hook should be attached to the point of spinal angulation and the head of the fetotome positioned next to it. During the sawing process the obstetrician should hold both the Krey hook and the fetotome with one hand and the handle of the instrument outside the cow with the other.

Once the cut is completed, the fetal 'halves' are carefully extracted, using the Krey hook. Sometimes, extra cuts may be needed to further reduce these fetal parts, as sharp bone fragments frequently result. To allow atraumatic delivery, the cow's birth canal should be protected when the fetal segments are delivered.

Alternatively, the wire may be passed around the spinal flexure of the fetus (Fig. 15.30). Passing the curved introducer around the rigid fetus may be a tedious task; using a smaller tool such as a wire handle may make this procedure easier. When accomplished, the fetotome is rethreaded, and the instrument passed into the vagina until its head abuts on the fetus. The fetal vertebral column is then cut through, and the smaller fetal segment is removed. Should difficulty arise over withdrawal of the remaining portion, it too may need to be divided perpendicularly to the first section, again using obstetrical wire.

When a schistosome presents by its extremities (i.e., three or four legs, with or without the head), the disproportionate diameter of the fetus, complicated by ankylosed joints, is likely to prevent natural or manipulative vaginal delivery (Fig. 15.31). Unless the fetus is very small in relation to the maternal pelvis – as might occur in case of a schistosome twin to a normal calf – time should not be wasted on an attempt at delivery by means of vaginal extraction. A Caesarean hysterotomy or fetotomy will be required instead. It is often possible and easier to deal with such a presentation by the latter method. A Caesarean hysterotomy will require a very large uterine incision and carries the risk of uterine rupture during extraction of the fetal monster, unless a fetotomy is carried out from the laparotomy site (Bezek & Frazer 1994) (see Chapter 16). If possible, the prethreaded fetotome is introduced, and the wire loop guided up and around all four limbs. When placed correctly, the wire will also encompass the head and parts of the thorax and pelvis (Fig. 15.32). A calving chain attached to one or two limbs can be used to anchor the fetus to the instrument. If the cut is successful, the head, neck, forelimbs, and part of the pelvis with

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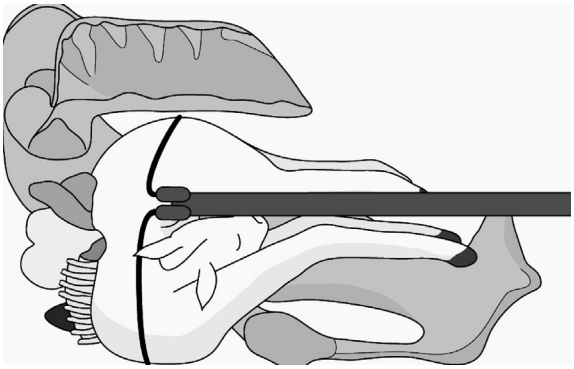

• **Fig. 15.32** Percutaneous fetotomy: position of the fetotome and wire to bisect a schistosoma reflexus calf presenting by its extremities.

hindlimbs attached can be withdrawn. Sometimes, the head and one or two limbs need to be removed separately to make birth possible. Another cut through the point of spinal angulation may be necessary to divide the remaining fetal trunk, so these parts can then be extracted safely without causing trauma.

After successful delivery of a schistosome, the uterus should always be examined closely for any injury and to ensure the absence of a second fetus.

Percutaneous Fetotomy in the Obstetrical Management of Perosomus Elumbus

This type of fetal monster is usually not very large and the forepart of the fetus is typically born without any assistance. Difficulty will be encountered when the obstetrician tries to complete the delivery because the hindlimbs are commonly ankylosed and twisted. Attempts to withdraw the fetus by traction may result in perforation of the uterus. Delivery can be accomplished by means of a partial fetotomy similar to that described previously for managing cases of 'hip-lock'.

Postoperative Management and Care

In many cases the dam requires less care after a fetotomy than after a Caesarean hysterotomy. Upon completion of a fetotomy, the fetal membranes should be removed only if they are detached. This is followed by a thorough lavage of the uterus with large volumes of warm water or a 'saline solution' (9 g of common salt/litre of water) at body temperature, using a wide-bore stomach tube. The fluids may be fortified with a povidone-iodine solution (<1 mL/litre of fluid) or other mild disinfectant. The warm saline solution is believed to have both a soothing and a stimulating effect on the uterus, and this, together with the excavation of uterine fluid, lubricant, and debris, may promote involution. Although some obstetricians advocate using cold water in order to stimulate uterine contractions, there is no evidence that this is of any real benefit. Fluids should be infused incrementally (4–5 litres at the time) and then siphoned out until the efflux is clear. Normally, 20 to 30 litres of water are needed, and it is important that approximately the same volume of fluid is siphoned out from the uterus as has initially been infused. Oxytocin (20–40 IU) and a nonsteroidal antiinflammatory agent are mandatory; these medications should be administered for 2 days. If there is evidence of shock, fluid therapy is also indicated – hypertonic saline solution (4–5 mL/kg) given intravenously followed by 20 to 25 litres of water orally. Intrauterine antibiotics are of limited value at this stage, but systemic broad spectrum antibiotics should be administered for at least 4 to 5 days, particularly in protracted cases. In addition, the administration of calcium borogluconate to facilitate uterine involution should be given due consideration. There is some anecdotal evidence suggesting that the uterus will contract and involute quicker if the cow is allowed exercise rather than being confined to a stall (Norman & Youngquist 2012). A transrectal examination of the entire genital tract should be carried out 2 to 3 weeks later, at which stage any adhesions can be easily identified. This is also the correct time to administer intrauterine antibiotics to treat chronic endometritis.

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Defects of Presentation, Position and Posture in Livestock: Delivery by Caesarean Hysterotomy

JOS J. VERMUNT, TIMOTHY J. PARKINSON AND DAVID E. NOAKES

Cattle

The Caesarean hysterotomy is one of the most common surgical procedures performed by veterinarians in cattle practice and is considered as a routine obstetrical technique. It has good maternal and fetal survival rates and is often less exhausting, faster, and safer than fetotomy (Parkinson 1974, Cattel & Dobson 1990). There are three main goals for doing Caesarean hysterotomy: (1) survival of the cow; (2) survival of the calf; and (3) maintenance of fertility. A prompt decision to perform Caesarean hysterotomy is important for optimum success (Dawson & Murray 1992). Ideally, it should be carried out when a live calf cannot be delivered after 15 to 20 minutes of manipulation. The cow is a good surgical risk, provided that the environment is suitable for aseptic abdominal surgery. Urgent intervention is indicated if there is evidence of fetal hypoxia, as shown by markedly increased movements of the fetus and expulsion of meconium, identifiable in the amniotic fluid. A successful outcome depends on several factors:

- Skill and speed of the surgeon
- Duration of dystocia
- Physical condition of the dam
- Availability of skilled assistance
- Surgical environment
- Concurrent disease
- Presence of a live calf

Indications

The reasons for surgery include most causes of dystocia, but analysis of published data shows that these six main indications account cumulatively for 90% of all Caesarean operations:

1. Fetomaternal disproportion (relative and absolute fetal oversize)
2. Incomplete dilation or induration of the cervix
3. Irreducible uterine torsion
4. Fetal monsters
5. Faulty fetal disposition (presentation, position, or posture) that is not correctable by vaginal manipulation
6. Fetal emphysema

In individual published series, their relative frequency of these indications varies considerably depending primarily on the breed of cattle predominantly at risk and, to a lesser extent, on whether fetotomy is routinely practiced. If the birth canal is fully dilated, fetal causes of dystocia may be amenable to relief by fetotomy, but failure of cervical dilation and irreducible uterine torsion are absolute indications for surgery. Nonsurgical delivery may seem advisable if the fetus is grossly infected, but laparohysterotomy is often obligatory in such cases because of premature uterine involution, emphysema of the fetus, or constriction of the birth canal. In many cases of dystocia, fetotomy may be the preferred option. However, the most important factor in choosing between Caesarean hysterotomy and fetotomy is likely to be the veterinarian's preference and relative experience with either technique (Schultz et al. 2008).

The indications for Caesarean hysterotomy and the reasoning behind an appropriate decision have been extensively discussed (Cox 1987, Pearson 1996, Green et al. 1999). In all cases the prognosis and cost should be discussed with the owner before surgery and, when necessary, written, informed consent should be obtained.

Fetomaternal Disproportion

Fetomaternal disproportion is consistently the most frequent overall indication for Caesarean hysterotomy in cattle. Four particular forms may be encountered.

Physical Immaturity of the Dam

In herds in which bull and heifer calves are kept together or in which a bull runs with suckling cows, calves may conceive at a surprisingly early age. It is not uncommon for heifers to be parturient at term when only 14 months old and, in exceptional cases, at only 1 year of age. Even at 18 months of age, the maternal pelvis is still immature and usually too small for normal vaginal delivery. This form of fetomaternal disproportion is also known as 'relative fetal oversize' (see Chapters 12 and 14).

Fetal Oversize

The majority of cases of disproportion are animals that are mature and at normal term. In this situation of 'absolute fetal oversize',

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the maternal pelvis is of normal size but the fetus is abnormally large, although normally developed in all other aspects. Among dairy breeds the Holstein–Friesian is more susceptible to this form of dystocia during the first pregnancy than the Ayrshire or Jersey. Certain beef breeds are also frequently affected with fetomaternal disproportion and not only during the first pregnancy. Double muscling or generalized muscular hyperplasia is well recognized in certain breeds such as the Belgian Blue.

The management of dystocia caused by fetomaternal disproportion depends largely on experienced clinical assessment of how much traction can safely be exerted without risk of serious birth canal trauma or, worse still, impaction of the fetus after only partial delivery. This is the most worrying of all obstetrical problems to be encountered in cattle practice, with ample scope for errors of judgment, which may often lead to death of the fetus and sometimes the dam. In many cases of fetal oversize, the fetal head cannot be drawn into the maternal pelvic cavity and the decision to perform a Caesarean hysterotomy is obvious. In others, traction is more effective and the decision is less clear. The difficulty lies in knowing when to stop traction in preference of surgery. Excessive force in such animals may merely exacerbate the degree of dystocia and compromise the success of a subsequent Caesarean operation. One practical guideline is that Caesarean hysterotomy is indicated if the head and both elbows in anterior presentation or both stifles in posterior presentation cannot be pulled into the pelvic canal using moderate manual traction by one person. As a rule of thumb, the elbows are introduced into the pelvic cavity if the fetlock joints extend for at least one hand width beyond the cow's vulva. Similarly, the stifles are introduced if the hock joints extend for at least one hand width beyond the cow's vulva. However, even then, if the calf has a large chest or pelvis, subsequent difficulty can still be encountered. The frequent finding in hospital referrals of fractured limb bones in oversized calves suggests either an unreasonable degree of traction or traction in a wrong direction. In heifers in particular, hurried traction early in second stage labour should be avoided unless there is obvious dystocia because the vestibule and vulva have not relaxed sufficiently and perineal damage is more likely to occur (see Chapter 14). If in doubt about the decision, it is probably better for the welfare of the cow and calf to perform Caesarean hysterotomy (Green et al. 1999). From an ethical point of view, the deliberate adoption of breeding policies that require delivery by means of Caesarean hysterotomy cannot be justified.

It is not uncommon for several heifers in a group to require more than normal assistance or delivery by Caesarean hysterotomy. If the time interval permits, the premature induction of parturition in the later calving animals within 10 days of the anticipated term may be of considerable benefit in such a situation (see Chapters 6 and 8). When elective surgery is required, it should ideally be performed during the first stage of labour.

Fetal Monsters and Infection

The most extreme form of disproportion is when there is pathological fetal oversize such as fetal anasarca and achondroplasia, which greatly increases the crossoperational diameter of the fetus. Conjoined twins are usually also too large for vaginal delivery. More common than all of these, however, is emphysema due to secondary putrefaction, which frequently develops in protracted cases of dystocia.

Postmaturity

A moderate prolongation of pregnancy up to 290 days or thereabouts is a normal feature of certain breeds, but in occasional animals of any breed, gestation may last for considerably longer, even beyond 400 days. There is continued fetal growth *in utero* with postmaturity, particularly of the skeleton. In such cases, dystocia at term is due not simply to fetal oversize but also to inadequate relaxation of the birth canal. There is also the problem of 'giant' calves associated with *in vitro* maturation/fertilization (Chapters 9 and 12).

Incomplete Cervical Dilation

Incomplete dilation of the cervix (see Chapter 13) is a common cause of dystocia in cattle; however, it should be diagnosed only after careful assessment of the findings on vaginal exploration. Cervical dilation during the first stage of labour is a gradual process and the presence of a cervical rim is not in itself an indication of dystocia, provided that the fetal membranes are still intact. Care should be taken in such cases not to perforate the membranes unless the cervix remains incompletely dilated 2 hours or so later. Slow or arrested dilation in multiparous cows may be associated with uterine inertia associated with parturient hypocalcaemia; in these animals, the response to calcium therapy is usually rapid. If, on initial or subsequent examination, the cervix is incompletely dilated and the membranes have already ruptured, with a fetal extremity presented against or through the cervix, or if the fetus is already dead, then further cervical dilation is unlikely to happen. If the cervical rim is shallow and membranous or if it stretches sufficiently for the head to be drawn into the vagina, then normal safe delivery may be possible. In the other cases, irrespective of the degree of dilation, the cervix is usually too thickened and indurated for a vaginal delivery to be safely attempted, and further delay will only result in fetal death and a greater risk of intrauterine infection. The presence of an incompletely dilated cervix after the birth of one twin, with the other fetus still *in utero* and often in a breech presentation, clearly indicates that the cervix starts to close again soon after becoming fully dilated. The frequent finding of faulty fetal disposition in cases of apparent failure to dilate may indicate that, in these cases at least, the cervix is in fact constricting and the cause of dystocia is fetal rather than maternal. Failure of the cervix to dilate or remain dilated is not uncommon in cases of premature calving and can result in the fetal head becoming trapped in the anterior vagina. Incomplete dilation of the cervix is an important complication of uterine torsion. After successful manipulative correction of the torsion, the cervix is often only partially dilated and seldom dilates further (Pearson 1971). In such cases the cervical rim may be substantial; however, it is usually thin and will stretch in response to traction on the fetus. Incising the cervical rim in the midline dorsally during traction may allow safe vaginal delivery. However, it should be remembered that the fetus (in cases of uterine torsion) may be larger than normal and that the cervical incision may tear, causing severe haemorrhage or uterine rupture.

Irreducible Uterine Torsion

Torsion of the uterus in cattle (Chapter 13) constitutes a major indication for Caesarean hysterotomy, either because the torsion is irreducible or because the cervix fails to dilate after vaginal correction. In most cases of postcervical torsion, the degree of cervical dilation and vaginal twisting permits the introduction of a hand into the uterus for manipulation of the fetus. However, if the torsion affects the cervical canal proper or uterine body (i.e., precervical torsion), the fetus is totally inaccessible, and such torsions are an absolute indication for Caesarean hysterotomy. In cattle, uterine torsion differs from all other causes of dystocia in that one or both of the fetal membranes usually remain intact even if the placenta

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• **Fig. 16.1** Schistosoma reflexus which presented viscerally. A skin pouch envelops the trunk, head, and limbs. (Courtesy Dr. Jakob Malmo.)

separates, unless they are deliberately perforated. The presence of fetal fluids thus protects the fetus and the uterus from infection; in this respect the condition carries a favourable prognosis. Torsion, however, may still have serious detrimental effects on the uterus. Rotation through 360 degrees is common, and even two or three complete revolutions of the uterus sometimes occur. The greater the degree of uterine rotation, the greater is the interference with the venous circulation within the uterus and its mesometrial and mesovarian attachments. The combination of uterine displacement and oedematous swelling of its wall may well result in perforation of the uterine body, especially by the fetal head. In extremely protracted cases a fetal extremity may impinge, through a uterine tear, on the urethra or segments of large intestine and, consequently, cause rupture of the urinary bladder or gut, respectively.

In most cases of uterine torsion, the prognosis is excellent, although paradoxically the surgery may be technically difficult, first because small intestine is usually displaced and impedes access to the uterus and second because the presence of fetal fluids may make the uterus difficult to handle and impossible to exteriorize for suturing. Perforation of the oedematous uterine wall can easily occur during the intraabdominal manipulation of the grossly distended uterus.

Fetal Monsters

Schistosoma reflexus is by far the most common gross structural defect in cattle. Occasional cases are born spontaneously without assistance, whereas others may be extracted with moderate and controlled traction. Most affected fetuses, however, cause dystocia because the characteristic angulation of the spinal column greatly increases the crossoperational diameter, although the body weight is usually less than normal for the breed. The fetus is presented in one of two ways: its exposed viscera may protrude from the vulva or three or all four limbs and head may lie in the vagina and can be felt to be attached to the misshapen trunk. The latter presentation may cause confusion, particularly in cases in which the appendages are enclosed in an inverted pouch of the skin, which is all that can be palpated (Fig. 16.1).

Schistosome cases occur sporadically in several breeds, sometimes as a twin to a normal fetus (Fig. 16.2), and may still be alive at delivery. It should be noted that they are seldom associated with protracted parturition, presumably because they cause obvious manifestations of dystocia. The dystocia is best relieved by fetotomy (see Chapter 15), although some veterinarians prefer to do a Caesarean hysterotomy. It must be realized, however, that longer than normal abdominal and uterine incisions are usually necessary

• **Fig. 16.2** Schistosoma reflexus twinned to a normal calf.

when a hysterotomy is carried out. Furthermore, extreme care is needed in manipulating the fetus from the uterus in order to avoid serious uterine tearing, which easily follows excessive traction. This manoeuvre may be facilitated by the lubricating effect of residual amniotic fluid. The prognosis after Caesarean hysterotomy is usually good; however, the cow should not be rebred to the same sire.

Cases of achondroplasia or bulldog calf deformity and anasarca or fetal dropsy cause dystocia due to widespread subcutaneous accumulation of tissue fluids, which greatly increases the crossoperational diameter of the fetus. This causes gross disproportion, irrespective of the body weight of the fetus; however, the body weight may also be considerably increased (see Chapter 9). Both congenital defects may also be associated with severe fetal ascites, placental oedema, and hydrallantois.

Lesions of the fetal central nervous system may cause muscle contraction of the limbs, which prevents normal extension in preparation for birth. Arthrogryposis, sometimes associated with torticollis and kyphosis (Fig. 16.3A and B), has been shown to result from viral infection (e.g., Akabane virus, bluetongue virus, Schmallenberg virus) of the dam during pregnancy and is also recognized as a genetic abnormality in the Charolais breed (see Fig. 16.3C). Because the muscle contraction fixes limb joints in either flexion or extension, depending on the joint, the condition is sometimes called ankylosis. However, the bones are not fused. Spina bifida is less common in cattle but causes similar contractions, usually of the hindlimbs only because the lesion is located in the thoracolumbar region. Fetal anencephaly and the deformity described as perosomus elumbus may also cause limb abnormalities. In most cases of muscle contraction, the musculature of affected limbs is palpably underdeveloped. The degree of contraction is often too severe for a successful attempted delivery. However, if the forelimbs in an anterior presentation can be brought into the vagina, traction may cause the flexed hindlegs to perforate the uterus below the pelvic brim. Conjoined fetuses occur occasionally with varying degrees of fusion. These monsters generally require delivery by Caesarean hysterotomy unless only the head is duplicated, in which case a simple or partial fetotomy will readily relieve the dystocia.

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• **Fig. 16.3** (A and B) Fetuses with varying severity of torticollis and muscle contracture of the limbs. (C) Congenital arthrogryposis of a Charolais calf.

Faulty Fetal Disposition

Provided that the cervix is fully dilated and remains so, most early cases of faulty fetal disposition can be corrected manually or relieved by relatively simple fetotomy. However, the loss of fetal fluids followed by uterine contraction often makes such manipulations difficult and time-consuming and more likely to result in rupture of the uterus. In protracted cases, constriction of the cervix may then cause the fetus to start to decompose and become emphysematous.

Fetal Emphysema

In cattle fetal emphysema is a frequent complication of protracted parturition and, irrespective of the primary cause of dystocia, is often the immediate indication for Caesarean hysterotomy. However, such cases should be assessed carefully and realistically before the surgery is undertaken because fetal putrefaction can seriously influence maternal survival afterwards. Hence fetotomy may be an alternative and frequently better option (see Chapter 15). Bacterial culture of such fetuses usually yields a heavy growth of coliforms or coliforms and clostridial organisms. The latter infection is associated with a high maternal mortality rate in the immediate postoperative period, possibly because of endotoxaemic shock. On cursory examination the clinical status of these cows may seem reasonable despite gross uterine distension; the pulse rate, however, is usually significantly raised and the animal noticeably quiet on handling. Such premonitory signs are likely to be followed, as soon as the uterus is incised, by the onset of rapidly deteriorating shock, which is sometimes fatal within 12 to 24 hours despite intensive supportive therapy. Experience suggests that coliform infection alone is less serious than clostridial putrefaction; however, preoperative differentiation is not possible. Despite the significant mortality rate in this group of cases, surgery may nevertheless be worthwhile if either fetotomy or slaughter is not an alternative option.

Miscellaneous Indications

Occasionally, animals are encountered with full cervical dilation and a normal-sized fetus in which the caudal part of the birth canal is too constricted for delivery, even after episiotomy (see Chapter 14). The condition is particularly associated with Friesian heifers. The natural termination of a pathologically prolonged pregnancy (postmaturity) may also be associated with absence of normal parturient changes in the vagina and vulva and thus a need for Caesarean hysterotomy.

Abortion in late pregnancy sometimes requires treatment by Caesarean hysterotomy for several contributory reasons such as incomplete birth canal dilation, cervical constriction, fetal deformity, and faulty fetal disposition. Such cases are uncommon, but nevertheless important, because they may be associated with specific zoonotic infections such as leptospirosis, salmonellosis, and possibly listeriosis.

Fetal mummification and hydrops uteri may now be treated initially by inducing parturition using prostaglandins, but a Caesarean hysterotomy may still be necessary if induction fails or the birth canal is insufficiently dilated for vaginal delivery. Neal (1956) described two-stage Caesarean hysterotomy for cases of hydrallantois. A left flank incision is made as for a normal Caesarean. Once the uterus has been identified, large-bore sterile tubing is used to drain the allantoic fluid through a stab incision in the uterine wall, with the tube being retained by a purse-string suture. The allantoic fluid is drained slowly as the heart rate of the animal is continuously monitored; if the rate increases, drainage is suspended for 10 to 15 minutes. When as much fluid as possible has been drained from the uterus, the tube is withdrawn and the purse-string suture closed. Alternatively, a rumen trocar plus cannula can be used, which is inserted through the skin and muscle layers of the right lower flank and then directly into the grossly distended uterus. Once the trocar is removed, allantoic fluid will flow from the cannula. Now, Caesarean hysterotomy can be carried out straight away rather than waiting 24 hours as originally described by Cox (1987).

Laparotomy is essential in cases of uterine rupture. If this disorder occurs as a preparturient complication, the fetus usually lies completely free within the abdominal cavity and, if the cord is not twisted, may survive until the placenta separates at term (a pseudoectopic pregnancy). More frequently, however, uterine rupture occurs as a complication of dystocia, particularly uterine torsion, or as a result of manipulation of a fetus that is oversized or has

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faulty disposition. Rupture of the uterus during parturition may result in considerable uterine haemorrhage and hypovolaemic shock.

Repeated dislocation of the sacrococcygeal articulation during assisted delivery in successive parturitions or a healed fracture of the maternal pelvis can result in deformation or a significant bony obstruction at the site, creating an uncommon indication for surgery.

Restraint, Preparation for Surgery, and Anaesthesia

One of the paramount goals of a Caesarean hysterotomy should be to limit the contamination of the peritoneal cavity with uterine contents. Peritoneal cavity contamination, particularly in cattle with dead, emphysematous fetuses, greatly increases the risk of peritonitis, limits the cow's chances of survival, and limits the surviving cow's productivity. Several different surgical approaches have been described for the Caesarean operation in both standing and recumbent cattle, with each approach having its own advantages and disadvantages (Schultz et al. 2008). These comprise the left paralumbar fossa celiotomy (standing or recumbent), right paralumbar fossa celiotomy (standing or recumbent), ventral midline celiotomy (recumbent), ventral paramedian celiotomy (recumbent), ventrolateral celiotomy (recumbent), and lateral oblique celiotomy (standing or recumbent). A low-flank celiotomy has been developed as an alternative approach for the delivery of an emphysematous fetus in the recumbent animal. Considerations crucial to the choice of approach include the following:

- The type of dystocia
- The surgeon's experience and confidence
- The nature of the environment (e.g., whether or not there is a crush or head bail for standing restraint)
- The availability of assistance
- The temperament of the animal
- The demeanour and physical condition of the cow (e.g., whether it can stand for the entire procedure, whether the rumen is distended, or whether there is evidence of calving paralysis due to prolonged dystocia)

The options of patient positioning for Caesarean hysterotomy are the following:

- Standing (suitable for left or right paralumbar fossa approach and lateral oblique approach)
- Dorsal recumbency (suitable for ventral midline and ventral paramedian approach)
- Sternal recumbency (suitable for left or right paralumbar fossa approach)
- Lateral recumbency (suitable for ventrolateral and low-flank approach)

The choice of positioning largely depends on the surgeon's preference and the demeanour of the animal, as well as available facilities. In a cow capable of tolerating surgery when standing, the left paralumbar fossa or flank approach is the standard technique for a viable or recently dead, uncontaminated fetus. Other indications for this approach include cows with a large udder, extensive oedema of the udder and ventral abdomen, or prolific ventral abdominal vascularity close to the milk vein such as occurs in mature dairy cows. One advantage of the left flank incision is that the rumen's anatomical position prevents exposure of the intestines. However, in individual cases, a large rumen, particularly if the animal is straining, may interfere with surgical access to the abdomen. Another advantage of the flank approach in the standing animal is easier correction of a uterine torsion. Finally, wound dehiscence is more manageable in the flank compared with lower abdominal incisions.

The standing left paralumbar fossa approach is the most commonly used approach for an uncomplicated Caesarean hysterotomy. In general, paralumbar fossa approaches are often preferred by large animal veterinarians because most of them are familiar with these. They are sufficiently similar to that used for rumenotomy and for either the right or left approaches for correcting abomasal displacement, so that most practitioners have a great degree of comfort with them. The standing left paralumbar fossa approach is described in detail later in this chapter.

Restraint, Facilities, Sedation, Assistance

For standing left paralumbar fossa surgery, the animal should be restrained in a calving pen using a halter tied in such a manner that the animal's right flank is against a wall and the head in the corner in order to limit movement during surgery. Alternatively, the cow can be restrained in a crush or head bail, which must provide good access to the left side of the animal. If needed, the animal can also be positioned against the exit gate of the left bail area in the milking parlour. A strategically fastened side rope should prevent the animal from turning around. The halter should be tied with a quick release knot in case the cow suddenly becomes recumbent. Nose grips may be required for additional restraint. A rope can be attached to the right hindleg above the fetlock and laid underneath the animal's body so that, if the cow becomes recumbent during surgery, the rope can be pulled to enable the animal to lie in right lateral recumbency. The tail should be tied out of reach of the surgery site, usually to the halter along the right side of the animal or to the animal's right hock.

Usually, the surgeon has little choice when selecting the environment for Caesarean hysterotomy. Many successful surgeries have been performed under less than ideal circumstances in pastures, barns, sheds, and milking parlours. If a hospital facility is not available, consideration must be given to choosing the cleanest possible surroundings, as the operating environment has been shown to be a significant source of organisms that may contaminate the wound. Also, the location for surgery should be selected carefully with the objectives of ensuring good lighting, facilities for restraint, and a suitable floor surface. Avoid doing surgery in buildings occupied by large numbers of other cattle. Ideally, use a clean calving pen or other unoccupied building. Clean bedding should be provided, although vigorous shaking of straw will cause unwanted clouds of dust. Adequate lighting should be available to illuminate the intended surgical site. The surgeon should ensure that the light is not situated such that the surgical site is in the shadow cast by the surgeon; equally, the light must not shine in the eyes and distract the surgeon. Many veterinarians carry a portable halogen lamp and stand for use on the farm; alternatively, the surgeon may choose to wear a head torch.

Facilities for restraint should be appropriate for the size of the animal and designed to avoid injury to animals or humans. A ring fixed in the wall of a calving box and offset 50 cm from the corner is ideal; if the animal goes down the offset ring encourages the animal to lie on her right side. The floor surface should provide adequate friction for animal and surgeon. Slippery concrete floors can lead to accidental falls during surgery. A 20 to 30 cm thick base of sand with clean straw on top provides an ideal surface. Facilities for the calf should also be prepared at this stage; a warm, straw pen complete with resuscitation equipment would be ideal.

Sedation should be avoided if possible because it can cause recumbency during surgery and may be detrimental to fetal survival. If sedation is deemed necessary, xylazine is commonly used (0.05–0.1 mg/kg i/m or half this dose rate i/v; however, the latter

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may not be a licensed route of administration in some countries). Unfortunately, xylazine has also a myotonic effect, causing uterine contractions, which make the uterus friable (Hoeben et al. 1997). Such contractions may make exteriorization of the uterus rather difficult and suture placement more problematic, increasing the likelihood of uterine tears during closure. Furthermore, xylazine can cause ruminal tympany, thus obstructing the surgical wound. If sedation is indicated, such as when cows struggle or remain fractious, it is advised to administer xylazine after the uterine closure is completed. Acetylpromazine maleate (0.05–0.1 mg/kg i/m or i/v) can also be used as a sedative, and it does not induce uterine contractions. However, it will take quite some time for this drug to take effect and the degree of sedation is relatively minor.

Alternatively, surgery may be performed on the recumbent animal; this is particularly indicated in case of fractious animals. If the cow is not already recumbent, a sedative can be administered (xylazine 0.2 mg/kg i/m, or 0.1 mg/kg i/v) or the animal may be cast using a rope. She should be placed in right lateral or semisternal recumbency with the body slightly tilted to the right. Bales of straw may be used to prop the cow in a stable position for surgery. In addition, the legs may be hobbled, although some surgeons prefer the left hindleg to be extended caudally and fixed by a rope.

Two or more assistants are usually required for successful surgery: as a minimum, one to restrain the cow and one to deliver the calf and provide appropriate care after the procedure. Communication with the assistants by the surgeon is important. Briefly describe how the surgery will be performed and outline the role of each assistant and how to proceed in the case of a crisis such as the cow becoming recumbent during surgery.

Anaesthesia

Regional anaesthesia may be accomplished by a variety of methods. The choice of anaesthetic method differs between surgeons and the selected surgical site. For flank incisions, paravertebral anaesthesia of the nerves associated with the transverse processes of T13, L1, L2, and sometimes L3 is commonly recommended. Each site is infused using 20 to 30 mL of a 2% lignocaine solution: around 15 to 20 mL to block the ventral nerve branches and 5 to 10 mL for the dorsal branches (Cox 1987). Signs of successful anaesthesia are a warm, hyperaemic and flaccid flank with no response to pain when tested with a hypodermic needle. The advantage of paravertebral anaesthesia is that the whole flank musculature is desensitized and flaccid, which facilitates exploration of the abdomen during surgery as well as closure of the wound. Also, the flank incision can be readily extended if necessary. One particular disadvantage is that the technique is more difficult to carry out than other methods, especially in fat or heavily muscled animals, in which case the landmarks are difficult to identify (Edmondson 2008). In addition, the cow may be unsteady after surgery because of loss of lumbar muscle tone and paresis of the ipsilateral hindlimb. Special care must be taken not to inject an excessive dose of anaesthetic solution around L3, as this spinal nerve contributes branches to the femoral and obturator nerves. Finally, the effect of vasodilation in the muscle layers causes a greater degree of haemorrhage that may require haemostasis.

A local anaesthetic line block and inverted–L block of the flank are excellent alternatives to paravertebral anaesthesia. An 18–gauge × 1.5–inch needle is used to administer 2% lignocaine hydrochloride at several sites, the number of sites being dependent on the length of the proposed incision. At each point, 5 mL of local anaesthetic is injected subcutaneously in each direction of the incision line and a further 10 mL into the musculature. Normally, only a total of 80 to 100 mL of lignocaine is required; larger volumes of local anaesthetic solution infiltrated into the incision area may have an adverse effect on wound healing. The technique is quick and reliable and requires minimal training. However, the parietal peritoneum may not be effectively anaesthetized, causing a reaction by the patient when it is incised. Sloss and Dufty (1977) reported particular problems of inadequate analgesia with an inverted L-block in fat animals. A similar reaction will occur if the incision has to be extended during surgery in order to extract the calf. Furthermore, because the flank is not flaccid, apposition and suturing of the muscle layers can be difficult.

High epidural anaesthesia or lumbosacral epidural block (anterior block), also using 2% lignocaine, can provide excellent anaesthesia of the flank, although such anaesthesia usually causes recumbency, which may be prolonged in cattle. Caulkett et al. (1993) reported that epidural anaesthesia using 0.07 mg/kg xylazine produced good analgesia for Caesarean hysterotomy in 45% of cases without causing severe ataxia. However, there was a prolonged time to the onset of anaesthesia, and it was not effective in 17% of cases.

Preoperative Preparation

Preoperative administration of antibiotics is strongly recommended (Cox 1987). Commonly, a broad spectrum antibiotic will be chosen, with ceftiofur sodium at 1 mL per 50 kg live weight being a good choice, as this antibiotic has no required milk withholding time. In beef cattle, long-acting tetracyclines could be administered. Tocolytic agents, such as isoxsuprine lactate (220–250 mg; when xylazine sedation is used, it is recommended to increase the dose by 100%) and the β_2–adrenoceptor stimulant clenbuterol hydrochloride (0.3 mg) administered by intramuscular or slow intravenous injection, are widely used and can facilitate the manipulation and exteriorization of the uterus during surgery. Furthermore, these tocolytic drugs counter the myotonic effect of xylazine on the uterus. A low or caudal epidural injection (posterior block), using 1 mL/100 kg live weight of 2% lignocaine, is essential to alleviate abdominal straining (see Chapter 14). Unfortunately, severe tenesmus occasionally fails to be prevented by epidural anaesthesia. It should also be noted that too high a dose of local anaesthetic solution may result in ataxia and/or recumbency. In case of anterior presentation (with the head of the fetus entered into the pelvis), it is important to repel the calf back into the relaxed uterus after the epidural and tocolytic drugs have taken effect. This will reduce the chances of continuous straining and of the cow becoming recumbent during surgery. (Note: tocolytic drugs may have milk and/or meat withholding times which also apply to the calf when delivered.) Cows that are markedly dehydrated may need to be given fluid therapy before surgery, such as hypertonic saline solution (4–5 mL/kg) given intravenously, followed by 20 to 25 litres of water orally.

A wide surgical field should be prepared. Initially, dirt and dust should be brushed from the flank and back of the animal before the operative field is clipped or shaved. In case of a flank incision the entire flank should be clipped from below the transverse processes dorsally to just above the milk vein ventrally, and from the last rib to the hindleg, level with the tuber coxae. The skin should be prepared using a surgical scrub (7.5% povidone-iodine or 4% chlorhexidine gluconate solution), followed by surgical spirit. Sterile drapes should be applied; in the standing animal a large, moisture-resistant drape with a suitable window can be placed over the back of the cow and down the flank. A useful alternative in the field is to use a wide roll of plastic film wrapped around the cow's body leaving only the surgical site exposed.

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Surgeons and assistants should ideally wear protective surgical scrub suits, even in the field situation. Alternatively, a clean and disinfected apron can be used. Many practicing veterinarians prefer not to wear gowns and gloves, in which case the surgeon's arms (all the way up, including the armpits and shoulders) should be bare, washed, and scrubbed thoroughly. At all times, however, emphasis should be placed on thoroughly clean surgery. Due consideration should be given to wearing long-sleeved plastic gloves (rectal examination sleeves with the finger tips cut off, and the gloves held in place with elastic bands) and surgical gloves, particularly by those veterinarians who do not wear protective gloves for claw trimming and other work in cattle practice that causes gross contamination of the hands.

Operative Technique (Described for a Right-Handed Person)

The adequacy of anaesthesia should be carefully tested before surgery because muscles and peritoneum may remain sensitive despite skin desensitization.

In all situations it is important to exteriorize the uterus. This helps to limit the contamination of the abdominal cavity, thus aiding in the prevention of peritonitis. The choice of surgical procedure has a direct bearing on whether the practitioner is able to exteriorize the uterus. Large fetuses, large cows, and small stature all limit the ability of the obstetrician to exteriorize the uterus.

Standing Left Paralumbar Fossa Approach

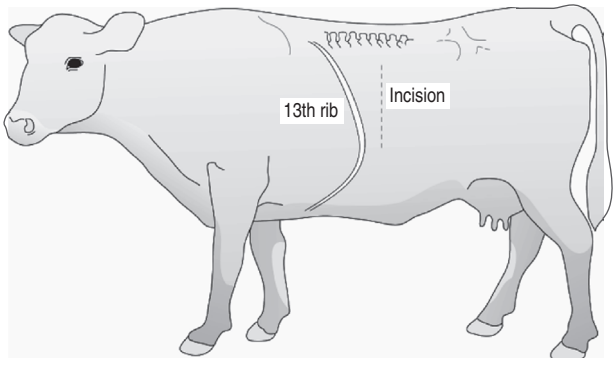
The left flank incision is the most common technique and most appropriate for the standing animal. The rumen helps to retain the abdominal viscera within the abdominal cavity. Absolute requirements for this approach include an appropriate restraint facility and a cow capable of standing through the entire procedure. The surgeon has to judge whether the animal will remain standing during the procedure; if not, recumbency should be induced before surgery.

A clear contraindication for this approach is a large fetus that will prevent exteriorization of the uterus. In such cases, lifting the uterus plus calf towards the paralumbar incision will be difficult and sometimes impossible for some veterinarians.

After preparation of the flank and appropriate anaesthesia for the incision site (Fig. 16.4), a vertical skin incision is made in the middle of the left flank starting 10 cm ventral to the transverse processes and extending approximately 30 to 40 cm long (Fig. 16.5). If the breed of dam or other indication for surgery suggests that a future elective Caesarean hysterotomy(s) may be necessary, the incision should be made at close to cranial border of the flank just behind the last rib, thus allowing for subsequent incisions more caudally.

With the left flank approach these muscle layers are incised: cutaneous, external abdominal oblique, internal abdominal oblique, and the transverse abdominal muscle. The external and internal abdominal oblique muscles are sharply incised in the same direction using a scalpel. Haemorrhage from these muscle layers is usually minimal; however, when large vessels are involved, haemostats should be applied and the vessel ligated if necessary. The aponeurotic transverse abdominal muscle is split vertically by blunt dissection. The peritoneum is tented and incised at the dorsal aspect of the incision using a scalpel, taking care not to puncture the rumen, which lies immediately beneath the peritoneum. At this stage, entry into the abdominal cavity is obtained, which is usually signalled by the sound of air rushing into the potential space. The incision is then extended vertically with blunt-tipped scissors rather than a scalpel, so as to reduce the risk of cutting abdominal organs. Often, a variable amount of peritoneal fluid, sometimes blood tinged, is immediately apparent in the abdominal cavity. Greater volumes are present in cases of prolonged dystocia, uterine infection, torsion, or rupture. Additionally, in case of uterine torsion or uterine infection, there may be large fibrin clots present in the abdomen. In case of uterine torsion the small intestines may also be displaced to a position immediately caudal to the rumen to such an extent that loops may spill through the abdominal incision. The surgeon should explore the abdomen to identify the uterus, and the uterine tone and disposition of the calf should be noted. Frequently, the tip of the pregnant horn is

• **Fig. 16.4** Preparation of the flank for surgery: A wide surgical field is clipped from the transverse processes dorsally to just above the milk vein ventrally and from the last rib to the hindleg, level with the tuber coxae. When paravertebral anaesthesia is being used (as here), the prepared area is extended to include the dorsal surface of the transverse processes. (Courtesy Lisa Whitfield, Senior Lecturer, School of Veterinary Science, Massey University.)



• **Fig. 16.5** Site for Caesarean hysterotomy: left paralumbar fossa approach – standard incision used in the standing or recumbent animal.

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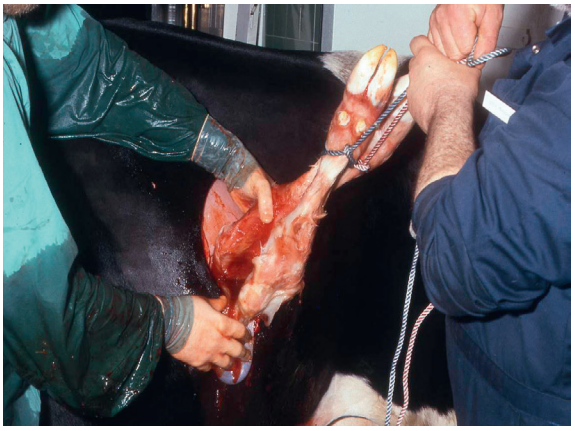
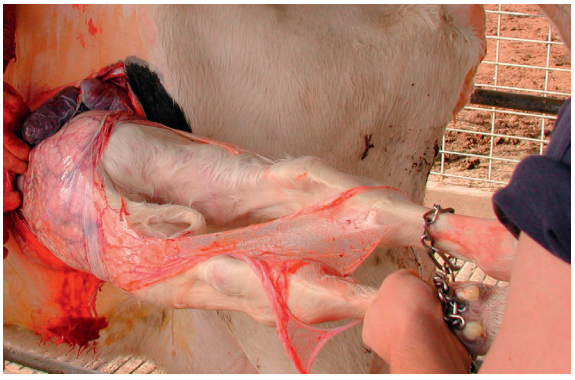
• **Fig. 16.6** Exteriorization of the fetal hock. The left hand cups one or both hocks while the right hand holds the digits in tip of the horn, which is pulled into the incision. The hock is then lifted, so levering the lower limb through the incision, and finally locking the hock in the ventral commissure of the flank incision. (Courtesy Dr. Jakob Malmo.)



• **Fig. 16.7** Removal of the calf in anterior presentation through the cow's left flank incision. Initial traction is dorsal and slightly lateral.



• **Fig. 16.8** During delivery of the calf's hips, the body is rotated and traction is directed caudally and laterally, at the same time rotating the calf in a manner similar to a vaginal delivery of a calf in posterior longitudinal presentation. (Courtesy Dr. Jakob Malmo.)

located in the left lower quadrant of the abdomen, close to the flank incision.

Calf in Anterior Presentation

The hindlimbs of the calf are situated in the tip of the pregnant uterine horn. With the calf in anterior presentation, it is ideal to anchor one or both hindlimbs in the incision, which holds the uterine horn outside the abdomen. Rather than attempting to lift the entire uterus, a common method to exteriorize the gravid uterus is for the left hand to first locate the calf's digits, then follow the metatarsus down to finally cup one or both hocks. Then the right hand grasps the digits in the tip of the horn from behind. The limb is now rocked towards the incision, and the tip of the horn is lifted through the incision. The hock is then elevated, so levering the lower limb up through the incision. Often, it is then possible to wedge the hock into the ventral aspect of the incision, while the digits are retained by the flank above the dorsal aspect of the incision (Fig. 16.6), thereby relieving the tension on the surgeon's arms. (Note: The tip of the uterine horn should not be grasped without including one of the calf's limbs. Attempts to manipulate or grasp the uterus, rather than the calf, will often cause uterine tears and abdominal contamination.)

Exteriorization of the uterus before making the incision is a critical step in the subsequent success of the surgery. However, traction on the uterus may require considerable strength and tenacity on the part of the surgeon. Manipulation of the uterus also stretches the mesometrium and can cause pain, which is manifested by the cow displaying signs of discomfort. Furthermore, once the uterus is being handled the myometrium often starts to contract, making exteriorization more difficult unless a tocolytic agent has been administered before surgery.

The uterine wall is incised over the calf's leg using a scalpel or scissors. The incision needs to be made large enough to allow removal of the calf without further tearing of the uterus or the need to extend the uterine incision. As a general rule, an incision from the tip of the digits to either the metacarpus or metatarsus is sufficiently long to permit extraction of the calf without causing uterine tears. If the incision in the uterus is made too short, the uterus may tear uncontrollably during extraction of the calf. The incision should be made over the greater curvature of the uterus, parallel to the longitudinal muscle layers of the myometrium, and distant from both the cervix and very apex of the pregnant horn. This area has the fewest large blood vessels. The greater curvature runs between and parallel to rows of cotyledons, which are both visible and palpable through the uterine wall. If these are incised inadvertently, they must be ligated to avoid substantial blood loss. If the uterine incision extends too close to the cervix, its suture repair will be very difficult. Also, care should be taken to avoid incising the calf, particularly if fetal fluids are sparse.

The membranes (allantochorion and amnion) are ruptured manually, and the calf's fetlocks are grasped by the surgeon, exteriorized, and passed to an assistant. Alternatively, sterile calving chains can be placed around the calf's hindlimbs and handed over to an assistant, who initially pulls the calf up in a dorsolateral direction until the calf's pelvis emerges from the flank incision (Fig. 16.7). Then lateral traction is applied such that the calf is rotated and removed in a manner similar to a vaginal delivery of a calf in posterior longitudinal presentation (Fig. 16.8). At the same time, the surgeon attempts to retain the uterus outside the abdomen in order to curtail contamination.

Exteriorization of the pregnant horn can be difficult and is not always possible, for example when the uterus is firmly contracted (even when tocolytic drugs have been administered). A large fetus and lack of physical strength may also make exteriorization more difficult. In these cases, incising the uterus has to be done within

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the abdominal cavity because the uterus has become friable or is liable to damage by further manipulation. However, such an incision may lead to gross contamination of the abdominal cavity with fetal fluids, which are unlikely to be sterile, particularly if dystocia was the indication for surgery. Furthermore, incision within the abdomen is often made more difficult by bouts of straining by the dam. This 'blind' incision should be made on the cranial aspect of the greater curvature of the pregnant horn by using a Robert's fetotomy knife (see Fig. 15.1) or a scalpel blade, in which case the surgeon's fingers should shield its cutting edge. Often, it is impossible to make a long enough incision, and it is safer to first make a small incision, then grasp one or both limbs of the calf through the incision and pull these into the flank incision. While an assistant applies gentle traction to the calf, the uterine incision can be carefully extended further.

A live calf should be attended to by an assistant while the surgeon examines the uterus, initially for the presence of a second fetus. In addition, any lacerations of the uterine wall should be noticed and repaired. Fetal membranes should only be removed at the time of surgery if they are lying free within the uterus or can be easily detached, which is uncommon. Otherwise, they are returned to the uterine lumen. Any protruding tags are trimmed so as not to incorporate these in the suture line of the uterine incision. This approach is justified on two grounds. First, it should be assumed that, if fetal membranes can be physically separated, they will be expelled naturally and more completely by uterine contractions. Second, if deliberate detachment of fetal membranes is attempted before they would normally separate and be expelled, then there may be haemorrhage or incomplete removal, either of microvilli or of larger masses of placental tissue. It is common practice to place antimicrobial pessaries in the uterine lumen before final closure of the hysterotomy wound, but the value of this is questionable. If fetal membranes are subsequently expelled naturally, so are the pessaries. If they are retained, then the antimicrobials will have no more than a minimal local effect in the lumen and are probably ineffective in controlling deep infection.

Calf in Posterior Presentation

Adequate rotation of the uterus (if required) is a prerequisite for success (see later in this chapter, under 'Rotation of the Calf'). Delivery of the calf through the abdominal incision is similar to that for a normal anterior longitudinal presentation. In case of posterior presentation the head and forelimbs of the calf are situated in the uterine horn. Often these structures together are so bulky that it is difficult, if not impossible, to get sufficient grip to elevate the pregnant horn and bring it into or near the incision. This technique is therefore recommended: With the left hand, the digits of the calf's underlying (most ventral) forelimb are grasped from above, and traction is applied so the tip of the uterine horn may be elevated high enough to bring the greater curvature into sight. An incision of about 15 cm can now be safely made with the right hand. The next step is for the left hand to grasp the metacarpus through the incision. Because this grip is firmer, the tip of the uterine horn can be elevated enough to allow the incision in the greater curvature to be extended, making sure to avoid cotyledons. The membranes are ruptured and chains applied to the forelimbs, which are handed over to an assistant who initially pulls the calf upwards. At the same time, the surgeon manipulates the head through the uterine incision. A finger and thumb grip in the orbits is often helpful in manoeuvring the head through both the uterine and abdominal incisions. Once the head and shoulders are outside the flank incision, the calf is slowly extracted by lateral traction. Often, there is a small amount of inconsequential uterine fluid spillage into the abdomen.

Occasionally, the hindquarters of the calf may still be wedged within the vagina such that vaginal repelling by an assistant is required. If so, the part of the calf protruding from the vulva (i.e., most of the hindlimbs) should be properly cleaned and lubricated before delivering the calf through the flank incision. The fact that many cows urinate immediately after removal of such a presented fetus suggests that urine has been retained because of compression on the animal's urethra.

Rotation of the Uterus

If the tip of the pregnant horn (left or right) is located in the right lower quadrant of the abdomen, it will be necessary for the surgeon to rotate the uterus along its longitudinal axis (clockwise) in order to bring the calf's limbs towards the flank wound (Schuijt & Van der Weijden 2000). This may be quite difficult to achieve in case of a big fetus or a surgeon of small stature. However, it is essential that the uterus is rotated because it allows the pregnant horn to be exteriorized. The uterus should be incised as close to the tip of the horn as possible, not over the upper part of a fetal limb situated in the body of the uterus. Incisions made in or near the body of the uterus usually can only be sutured within the abdominal cavity, which is extremely difficult.

There are two techniques for rotating the uterus such that the tip of the pregnant horn will come within reach and can be exteriorized as described previously:

1. Traction should be applied on a leg with the left hand while pushing the dorsal aspect of the uterus away from the surgeon with the palm of the right hand. Alternatively, both hands should be put against and under the back of the calf (which is situated in the body of the uterus, against the greater curvature, and facing towards the surgeon). The uterus is then elevated and pushed/rolled over to the other side of the abdomen. The same technique can be attempted if the indication for Caesarean hysterotomy is irreducible uterine torsion. The uterine incision can then be made after the torsion has been corrected. However, in cases of irreducible uterine torsion, the uterine wall is often oedematous and friable; thus extreme care must be taken not to perforate the uterus with the fingers.
2. The surgeon's right hand and forearm are brought beneath the body of the uterus such that the hand can be placed on the dorsomedial (upper) aspect of the tip of the pregnant horn. With the hand so positioned, the uterus is rotated about 90 degrees by pushing and pulling the tip of the horn underneath the uterus while lifting and thrusting the body of the uterus towards the opposite side of the abdomen using the surgeon's forearm and elbow.

Closing the Uterine Incision

Before closing the uterine incision, the uterus should always be checked for the presence of a second calf. The edges of the uterine incision are inspected for haemorrhage, particularly from cotyledonary vessels (Fig. 16.9). It is advisable to exteriorize both uterine horns before the genital tract begins to involute, as this will facilitate inspection and repair of the incision. Large vessels that are bleeding should be ligated. The uterus is supported by an assistant or held using uterine forceps (Fig. 16.10A and B) such that the entire uterine incision is kept outside the flank incision. The uterus is closed with absorbable monofilament suture material, such as chromic or plain catgut (6 USP or 10 metric), polyglycolic acid, and polyglactin 910. Studies have investigated

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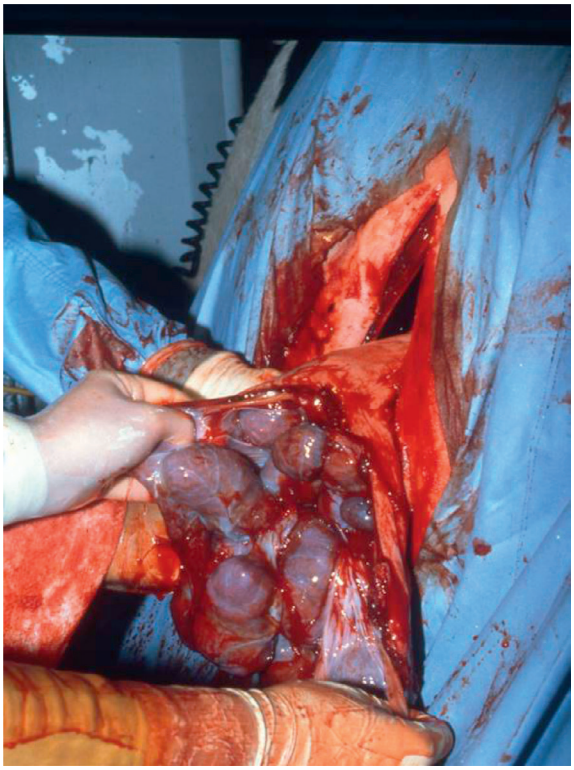
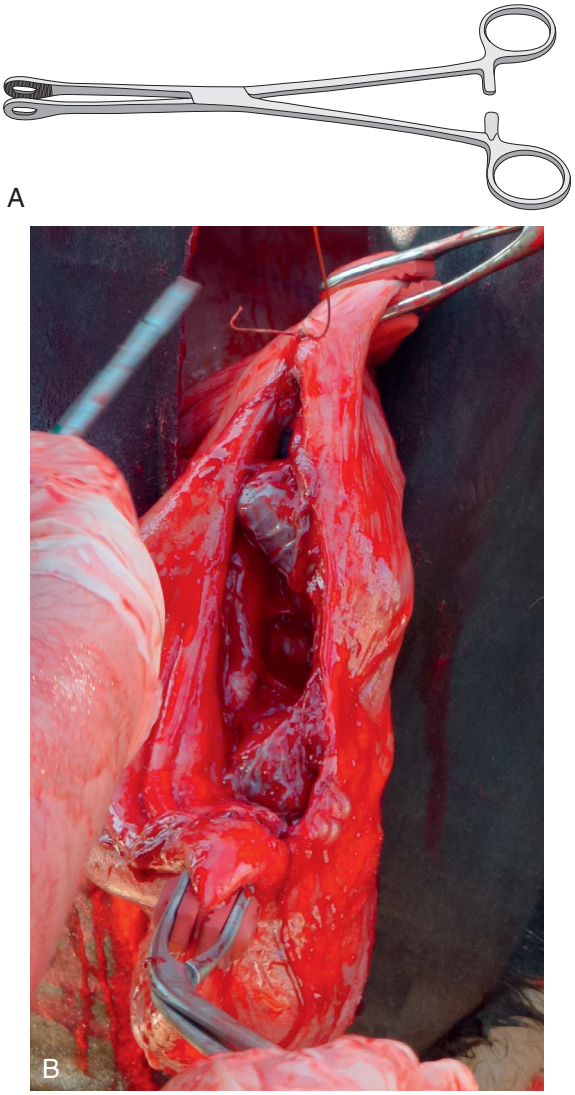


• **Fig. 16.9** Closing the uterine incision. The edges of the uterine incision are inspected for haemorrhage, particularly from cotyledonary vessels.



• **Fig. 16.10** (A) Uterine forceps for an assistant to hold the uterus (B) when the Caesarean incision is sutured by the surgeon.

uterine healing when a number of different suture materials were used. Surprisingly, large sized plain catgut is probably the suture material of choice. Synthetic absorbable sutures persist longer in tissues and have been associated with more dramatic scarring of the uterus (Newman & Anderson 2005). Additionally, catgut has advantages over the synthetic suture materials, particularly when the uterus is friable, because the latter may have a 'cheese wire' effect, making them more prone to cause uterine tears when tension is placed on the suture line. Braided suture materials seem particularly prone to cause tearing of the uterus as they are tightened. On the other hand, catgut causes greater tissue reaction and therefore is more likely to produce adhesions. The suture material and a large round-bodied (tapered) needle should be prepared for use before starting the surgery.

Suturing should start at the cervical end of the uterine incision, because this part of the uterus will retract into the abdominal cavity once the uterus starts to involute. A variety of suture patterns have been employed; all are continuous inverting patterns with the objective of creating a watertight seal by apposing the serosal surfaces while causing a minimum of subsequent adhesions and uterine scarring. The Utrecht method (Fig. 16.11), which is a modified Cushing pattern turned to a 30 to 45 degree angle away from the incision, is routinely used these days. The suture should start approximately 2 cm above the upper end of the incision, using oblique bites, so the knot will be buried within a fold of the inverting tissue. Similarly, the continuous interlocking, inverting pattern is inserted using oblique bites. These should begin about 2 to 3 cm from the incision edge but are inserted some distance back from the emerging previous bite (Fig. 16.12A). Each bite should exit the uterine tissue near the edge of the incision and then be pulled tightly. It is important not to perforate the uterine wall; sutures should be placed only partial thickness, incorporating the serosa and muscular layer of the uterus. Great care should be taken to avoid fetal membrane tags becoming incorporated in the suture line. The above mentioned suture pattern approximates the serosal surfaces, with the wound edges not inverted too much (uterine healing occurs across the wound edges rather than on the apposed serosal surfaces). The final knot is buried in the same manner as the knot at the beginning of the suture line. If done correctly, there will be no leakage of uterine fluid and little, if any, exposed suture material. Exposed suture material forms a nidus for adhesions to develop after intraabdominal surgery. If there is any leakage, the incision line should be oversewn with a second layer.

The advantage of this suture pattern is minimal adhesion formation after surgery. A single layer is usually sufficient, and this pattern is particularly efficient if the uterine wall is flaccid during repair of the incision. Alternatively, a Lembert suture pattern can be used with the needle passing at right angles to the incision, or a Cushing pattern, in which the needle passes parallel to the incision. Regardless of the suture pattern used, a rapidly involuting

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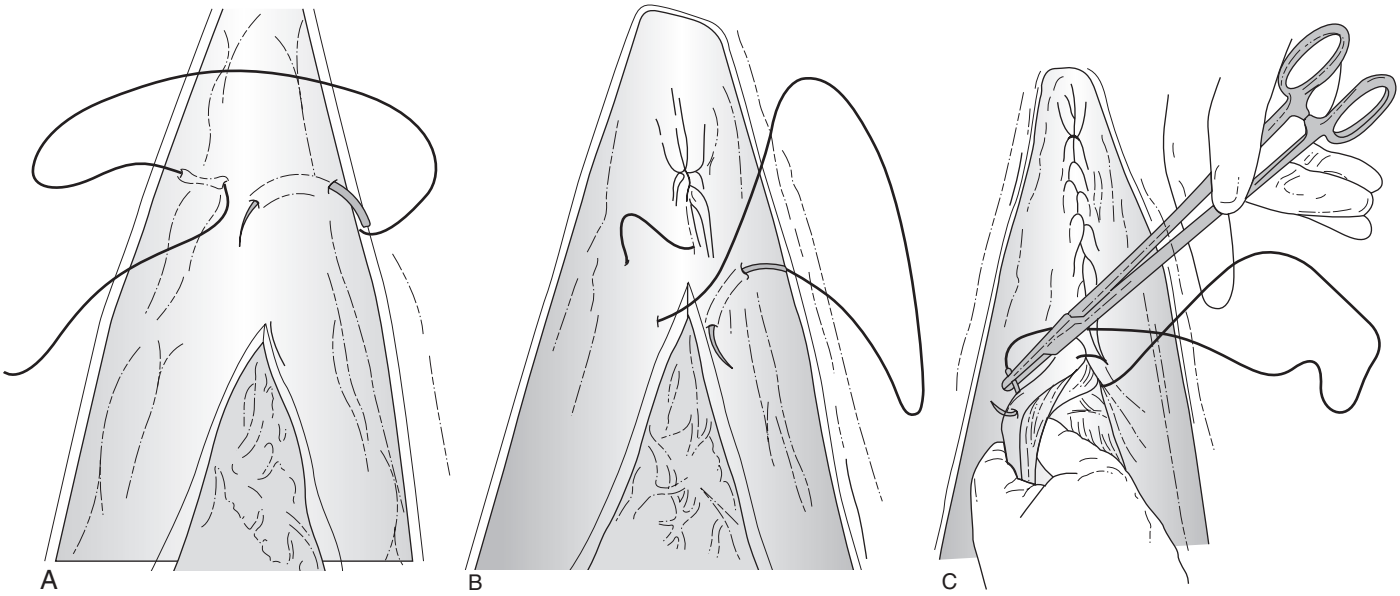

• **Fig. 16.11** Utrecht uterine suture technique. (A) The initial knot is buried in a fold of uterine wall. (B) The round-bodied needle is directed at an angle oblique to the incision. (C) This suture pattern produces a continuous interlocking, inverting, watertight repair. (Adapted with permission from Turner AS, McIlwraith CW. *Techniques in Large Animal Surgery,* 2nd ed. Philadelphia: Lea & Febiger; 1989.)

uterus means there will be less tissue in each bite of suture material than at the time of suturing, which potentially may loosen the suture line. Many surgeons oversew the first suture line with a second continuous pattern, particularly if the uterus is friable and where the suture material has the tendency to tear through the tissues.

Once the uterine incision has been closed and the uterus checked for any tears, the surface is rinsed with sterile fluids (e.g., Hartman's solution) to remove any blood clots and other debris. The uterus is then returned to its normal position within the abdomen, ensuring that there is no twisting of the genital tract (see Fig. 16.12B). At this point, oxytocin (20–40 IU) may be administered intramuscularly to hasten uterine involution. However, uterine contractions induced by oxytocin may incite abdominal straining, which will make suturing of the flank incision more difficult. Often, it is better to wait until the surgery is completed. The abdominal cavity is inspected for large blood and fibrin clots; if present, these should be carefully scooped out by hand. The administration of a water-soluble antibiotic, such as crystalline penicillin, within the abdominal cavity is recommended by some surgeons, but not others (Cox 1987). Metronidazole should not be administered because its use is prohibited in food-producing animals in Europe and many other countries, despite having been recommended by some surgeons (Dawson & Murray 1992).

The intraabdominal use of carboxymethyl cellulose (CMC) has been studied in sheep and recommended for prophylaxis against postoperative uterine adhesions (Moll et al. 1992). Nowadays, CMC is widely used in abdominal surgery in large animals, particularly horses, to prevent the formation of bowel adhesions. However, its effectiveness as an adhesion prevention compound after Caesarean hysterotomy in cattle has yet to be proven.

Closing the Flank Incision

The peritoneal cavity should be closed as quickly as possible to reduce the chance of bacterial contamination. The abdominal flank incision is repaired in two layers: peritoneum and transverse abdominal muscle in the first layer, and internal and external abdominal oblique muscles in the second. A simple continuous suture pattern is used, starting at the ventral commissure of the incision. Care is taken to fully appose the peritoneum and transverse abdominal muscle to avoid leakage of air from the abdominal cavity into the muscle layers after surgery (Sloss & Dufty 1977). Air leakage is not as likely after surgery on a recumbent animal because less air has been sucked into the abdominal cavity during surgery. The amount of air present within the abdomen can be reduced by an assistant compressing the opposite ventral abdomen and flank immediately before closing the upper aspect of the peritoneal incision. Sutures should be placed approximately 1 cm apart, using absorbable monofilament suture material (e.g., chromic catgut, 3 USP or 7 metric). When suturing the muscle layers, more tissue of the internal abdominal oblique muscle should be incorporated in each bite, i.e., the needle should enter the external abdominal oblique muscle closer to the edge of the incision than is the case for the internal abdominal oblique muscle. This technique will ensure good apposition of the two muscle layers (Fig. 16.13). To reduce dead space between the suture lines, deeper bites with the suture can be made periodically into the deeper suture line (that of the peritoneum and transverse abdominal muscle), thus intermittently anchoring the suture into the underlying tissue. Infusing tissue friendly antibiotics between the suture layers may be considered. Before closing the skin, it is advised to place several simple interrupted tension sutures wide and deep through the full thickness of the abdominal muscle layers.

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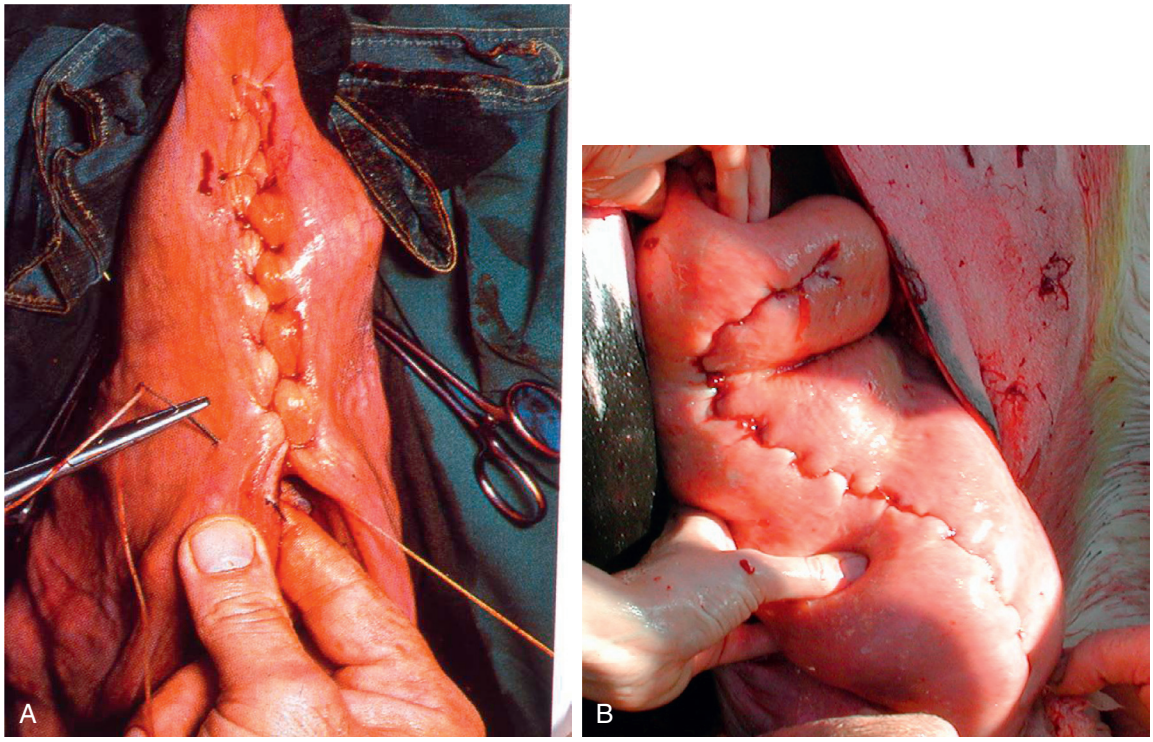

• **Fig. 16.12** (A) Placing the Utrecht pattern of sutures. The continuous, interlocking, inverting pattern is inserted using oblique bites that begin about 2 to 3 cm from the incision edge and that are inserted some distance back from the emerging previous bite. (From Kersjes et al. (1985) with permission.) (B) The repaired hysterotomy incision. (Courtesy Dr. Jakob Malmo.)

• **Fig. 16.13** Closure of the abdominal wall. (Courtesy Dr. Jakob Malmo.)

The skin incision is closed in a standard fashion, such as a simple interrupted horizontal mattress or cruciate pattern (Fig. 16.14), using a cutting needle and nonabsorbable suture material such as sheathed monofilament nylon (3 USP or 6 metric). Moderate tension should be applied to the sutures to bring the wound edges into complete apposition. This will ensure a good seal and promote first intention healing. In case of infection occurring the most ventral suture can then be removed to allow drainage and/or flushing of the wound. Although often described, the use of a continuous suture pattern, such as the Ford interlocking pattern (Fig. 16.15), is not recommended by the authors because of its associated risk of the suture line coming undone. Nowadays, this risk is markedly increased because the majority of cows are kept in loose housing systems or not housed at all.

Standing Lateral Oblique Approach

An alternative to the left paralumbar fossa approach in the standing cow is the left or lateral oblique approach, involving a slightly oblique incision from caudodorsal to cranioventral, about 45 degrees from vertical. The incision is started about 10 cm cranial and 10 cm ventral to the tuber coxae, extending cranioventrally to end about 3 cm caudal to the last rib (Fig. 16.16). The advantages of an oblique incision are that the internal abdominal oblique and transversus abdominis muscles can be split in the direction of their respective fibres and there is improved access to the genital tract (Cox 1987). Potential disadvantages are incision of the circumflex iliac artery if the incision is extended too far caudodorsally and lack of anaesthesia if too far cranioventrally when using paravertebral anaesthesia.

The abdominal wall incision should be closed in three layers, each of them in a simple continuous pattern. The peritoneum and

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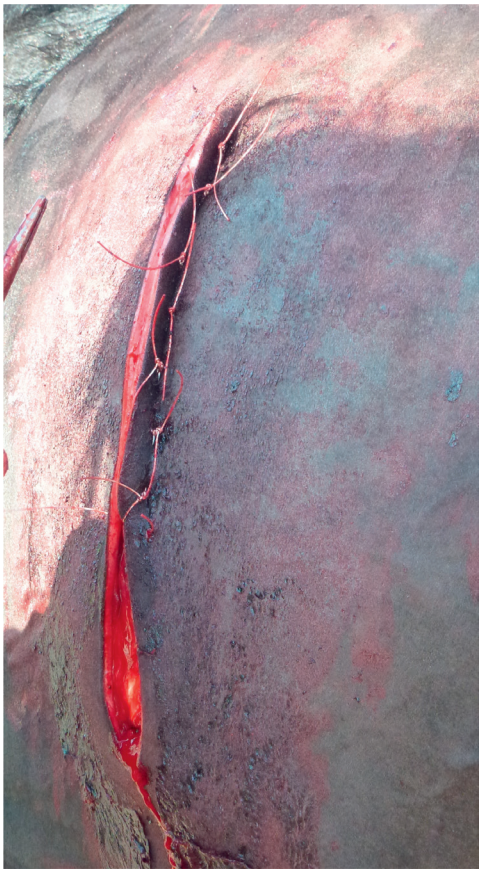
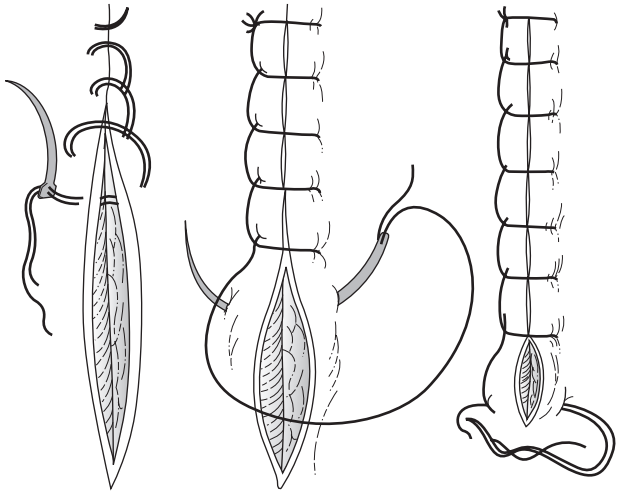


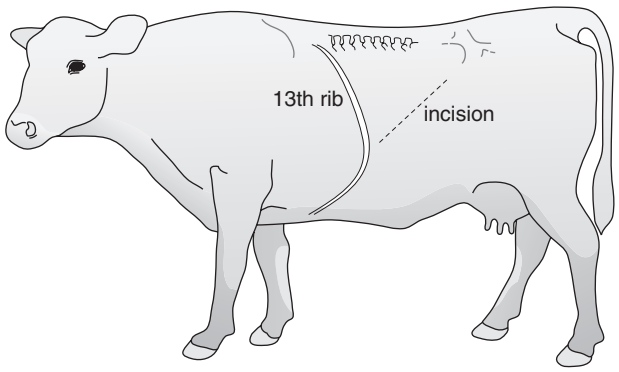
• **Fig. 16.14** The best means of closing the skin incision is by interrupted or mattress sutures. (Courtesy Lisa Whitfield, Senior Lecturer, School of Veterinary Science, Massey University.)



• **Fig. 16.15** The Ford interlocking suture pattern for the skin. (Adapted with permission from Turner AS, McIlwraith CW. *Techniques in Large Animal Surgery,* 2nd ed. Philadelphia: Lea & Febiger; 1989.)

transversus abdominis muscle are incorporated in the first layer, whereas the internal and external abdominal oblique muscles are sutured separately. Herniation is less problematic with this approach than with the ventral approaches (Schultz et al. 2008).

The lateral oblique approach allows better manipulation and far easier exteriorization of the apex of the gravid uterus than the standard flank or paralumbar fossa approach. It has been suggested that this approach is preferable for Caesarean hysterotomy in the standing animal with a dead fetus (Parish et al. 1995). It also has clear advantages for surgeons with either smaller stature or less physical strength.



• **Fig. 16.16** Incision site for Caesarean hysterotomy: left flank – lateral oblique approach, used in the standing or recumbent animal.

Standing Right Paralumbar Approach

The right flank incision is uncommon; however, it is indicated if the left flank approach is obstructed by adhesions as a result of previous surgery. Although access to the uterus is good, the small intestine is difficult to retain within the abdominal cavity and interferes with the surgery. Most veterinarians purposefully avoid this approach; however, some surgeons believe that right horn pregnancies are more manageable using the right paralumbar approach. This approach may be helpful when a large calf can be palpated in the right horn with its limbs directed towards the right side of the animal and in cows with a life-threatening hydrops condition of the uterus (Schultz et al. 2008).

Recumbent Left Paralumbar Approach

This approach varies little from the standing left paralumbar approach. However, additional assistance will be required to cast the cow, if not recumbent already, and to position the animal in right lateral recumbency. The incision is made somewhat more ventral than in the standing left paralumbar approach. Exteriorization of the pregnant uterine horn is often quite demanding because the gravid uterus tends to fall away from the incision. Closing the flank incision is also more difficult, due to increased tension on the muscle layers.

Recumbent Right Paralumbar Approach

This approach is rarely used, as it is very similar to that of the recumbent left paralumbar approach. It has the additional complication of not having the rumen present to retain the abdominal viscera within the peritoneal cavity (Campbell & Fubini 1990).

The various ventral approaches are discussed later in this chapter under the heading of 'The Emphysematous Fetus'.

Postoperative Care

Calf

The calf should be dried and the navel dressed with an antiseptic immediately after delivery. Once surgery is completed, 2 to 3 litres of the dam's colostrum should be administered to the calf using an oesophageal feeding tube if necessary. The dam should

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be introduced to the calf promptly, particularly in the case of a suckler cow and calf, to form a maternal bond.

Dam

The wound should be cleaned after surgery; how ever, no wound powder, spray, or antiseptics are applied to normal skin incisions. The teats and udder are examined, and oxytocin (20–40 IU) is administered intramuscularly to stimulate further uterine involution. In addition, calcium borogluconate should be administered intravenously to mature dairy cows to prevent hypocalcaemia and further promote uterine involution. The use of nonsteroidal anti-inflammatory drugs (NSAIDs) should be considered, at least in animals that have had severe dystocia, uterine torsion, or uterine infection before surgery. If there is evidence of surgical shock, the administration of intravenous fluids is indicated; 2 to 3 litres of hypertonic (7.2%) sodium chloride (4–5 mL/kg live weight) will be particularly effective. This should be followed by 20 to 25 litres of water given orally if the cow does not drink voluntarily. Systemic antimicrobial therapy should be directed towards the most common bacteria present in the postpartum uterus (i.e., *Trueperella pyogenes, Escherichia coli, Fusobacterium* spp. and *Prevotella* spp.) and should be administered for an appropriate period of time, usually 3 to 5 days or until the fetal membranes are expelled. Appropriate choices are procaine penicillin, ceftiofur, amoxicillin, ampicillin, and oxytetracycline. Many cows exhibit significant signs of pain after surgery (Kolkman et al. 2010) such that Barrier et al. (2014) recommended the routine use of meloxicam after surgery to promote a more rapid return of normal moving and lying behaviour patterns.

The dam is often reexamined 24 to 48 hours after surgery, at which time particular attention should be given to the animal's rectal temperature, demeanour, appetite, and faecal consistency. After surgery the faeces are often dry, and the cow may be mildly constipated. Pyrexia, depression, inappetence, and diarrhoea may indicate the onset of peritonitis. Normally, the faetal membranes are expelled 4 to 6 hours after delivery. However, appropriate treatment should be instituted if they are retained. Skin sutures should be removed no sooner than 3 weeks after surgery. In addition, a postnatal examination of the genital tract can be performed at the same time because endometritis is more common after Caesarean hysterotomy. Insemination should be delayed until more than 60 days after surgery.

The Emphysematous Fetus

Although fetotomy is generally the preferred procedure to achieve delivery of an emphysematous calf, a Caesarean hysterotomy is indicated when obstetrical manipulations or fetotomy is not possible. This may be because the uterus is contracted around the swollen calf, there is little or no uterine fluid present, the cervix is incompletely dilated or is constricting again, or the uterus is friable and therefore easily damaged. The veterinarian may also be inexperienced in carrying out a fetotomy.

In case of an emphysematous fetus, the main aim of Caesarean hysterotomy is survival of the dam; maintenance of fertility is of relatively little concern because of the life-threatening nature of the condition. Because it is often a salvage procedure, the welfare of the animal and cost to the owner are also important considerations. It is inappropriate to embark on a prolonged and costly Caesarean hysterotomy (or fetotomy for that matter) if the prospects for survival of the animal are poor. Hopeless cases or cases beyond the expertise and capabilities of the attending veterinarian should be recognized before the procedure is started and either the animal should be humanely destroyed or expert assistance sought.

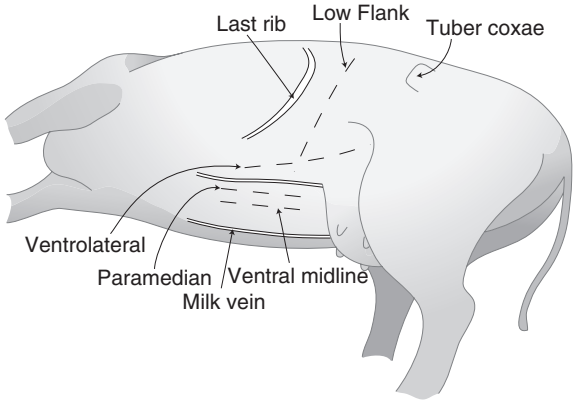
The most important rule in the management of an emphysematous fetus is not to panic. By the time the cow is presented, the question of how quickly the fetus is removed has little effect on the likelihood of the animal surviving. An emphysematous fetus is often associated with profound toxaemia and bacteraemia of the dam, resulting in fever, hypotension, dehydration, and shock. Therefore it may be necessary to first provide supportive treatment for cardiovascular stabilization and management of shock using intravenous fluids and NSAIDs.

In case of an emphysematous calf, exteriorization of the gravid uterine horn becomes crucial in order to avoid contamination of the abdominal cavity with infected uterine contents. In the standing animal, sufficient exteriorization is usually not possible and another approach (i.e., in the recumbent animal) should be used in which the uterus can be apposed more closely to the abdominal incision.

Recumbent Ventrolateral Approach

A ventrolateral incision is particularly indicated for the removal of an emphysematous fetus (Campbell & Fubini 1990). The gravid horn can be exteriorized more easily, but the incision may be difficult to close because of the tension on most tissue layers. Wound problems may also occur after surgery, such as breakdown and dehiscence or incisional hernia, because of the limited holding strength of the fasciae present in this part of the abdominal wall.

The cow should be cast in lateral recumbency with the pregnant horn nearest the incision. The hindlimbs are pulled caudally, and the upper hindlimb is abducted slightly to provide the best exposure to the incision site and more room in case the incision needs to be extended. An oblique abdominal incision in the lower flank, starting from the flank fold dorsal to the attachment of the udder, is continued cranially, parallel to the ventral border of the last rib; it more or less follows an imaginary line between the stifle and the navel (Fig. 16.17). In cattle with a large udder the incision can be extended caudally more easily than when a ventral midline or ventral paramedian approach is used. Therefore this ventrolateral approach may have particular value in dairy cows and older beef cows. Initially, the incision should be 30 to 40 cm long. When



• **Fig. 16.17** Alternative incision sites for Caesarean hysterotomy, all used in the recumbent animal and particularly suitable for extraction of an emphysematous fetus.

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necessary, it can be extended caudally towards the attachment of the hindlimb or cranially parallel to the subcutaneous abdominal vein (milk vein). As a precaution, this major vessel should be clearly identified and marked before the cow becomes recumbent, as it often collapses in recumbent animals.

The skin and external sheath of the rectus abdominis muscle are incised. The rectus abdominis muscle fibres are then bluntly separated in the direction in which they run. The internal rectus sheath is very thin, and its fibres are separated perpendicular to their orientation. The uterus lies directly underneath the incision, and an attempt should be made to exteriorize the entire gravid horn. If this is not possible, the abdominal wall should be pushed down and around the uterus. The uterus should be further isolated from the abdomen using drapes such as clean towels or plastic sheeting. The uterine wall is then incised and the emphysematous calf (with or without placenta) withdrawn from the uterus. After the removal of a grossly emphysematous fetus, the uterus is often noticeably ischaemic, of cardboard-like consistency, and totally atonic.

The uterine incision should be closed in a double layer, preferably using the Utrecht method, thus providing a secure closure, which is less likely to leak uterine fluids during contraction and involution of the uterus. The surface of the uterus (especially the suture line) should be thoroughly rinsed with an isotonic saline solution before returning it to the abdomen. The abdominal wall incision should be sutured in layers. The peritoneum and internal rectus sheath can be closed together. The rectus abdominis muscle is sutured (if possible) in a continuous pattern. The most important layer is the external sheath of the rectus abdominis muscle because it has the most tensile strength; it is best closed using a single interrupted or vertical mattress suture pattern. The skin is closed in a routine fashion. The integrity of the abdominal wall closure is less secure than that of either the ventral midline or ventral paramedian approach and therefore more prone to herniation (Schultz et al. 2008).

The advantages of the ventrolateral approach are that it gives good exposure of the uterus, even when it is friable, and that it minimizes the risk of uterine contents contaminating the abdominal cavity. However, repair of the abdominal muscle layers can be more difficult, particularly if the muscles are under tension, and sutures may tear through the tissues. A surgical drain may be inserted during repair of the wound.

Recumbent Ventral Midline Approach

The ventral midline approach (see Fig. 16.17) is not often used in the field because respiratory function may be compromised with the animal positioned in dorsal recumbency. Also, heavy sedation or general anaesthesia is sometimes necessary, and extra assistance will be required to cast and position the cow for this particular approach. Nevertheless, a midline incision provides excellent access to the uterus. Furthermore, the technique has the added advantage of the greater holding strength of the linea alba.

The cow should be positioned in dorsal recumbency, leaning towards the surgeon at a 30 to 45 degree angle, with both front- and hindlimbs secured to a post, gate, or wall. Such positioning is important because exteriorization of the pregnant uterine horn becomes difficult, if not impossible, if the cow is placed either in exact dorsal recumbency or leaning away from the surgeon. If the incision is properly made, the only body wall layers incised are the skin, subcutis, and the linea alba. When the peritoneal cavity has been opened, it may be necessary to pull the greater omentum cranially in order to adequately expose the uterus, after which the fetus is removed. Exteriorizing the uterus will be made easier by temporarily resting the hindlimbs flat on the ground, thus turning the abdominal incision closer towards the ground. After removing the fetus and suturing the uterus, the cow is returned into dorsal recumbency.

Closure of the abdominal wall is often problematic due to increased tension on the incision. Some surgeons prefer to close the linea alba in an everting, interrupted, horizontal mattress pattern using absorbable suture material such as polyglactin 910 (Schultz et al. 2008). Others choose to close the linea alba with braided, nonabsorbable suture material. Eversion of the linea alba allows the surgeon to oversew the linea with relative ease and safety. Other appositional suture patterns, including simple continuous and horizontal mattress, may also be used. No matter what suture material or pattern is used, the reliability of the abdominal wall closure is critical. Less than ideal closure may result in postoperative wound dehiscence, which has severe implications, including herniation or, in severe cases, evisceration. This approach should not be used when the large udder of older beef and dairy cows prohibits extending the midline incision sufficiently caudally to permit easier exteriorization of the uterus and when udder oedema and increased ventral vasculature make this particular approach more difficult.

Recumbent Ventral Paramedian Approach

In most aspects this approach is similar to the ventral midline approach (Turner & McIlwraith 1989). The abdominal wall incision is made parallel and approximately 5 cm lateral to the linea alba (see Fig. 16.17). However, the integrity of the abdominal wall closure may be less than that of the ventral midline approach, as neither the internal sheath of the rectus abdominis muscle nor the rectus abdominis muscle itself has substantial holding properties (Schultz et al. 2008). Also, closing a one-layer abdominal wall is easier and therefore preferable to a three-layer closure.

Recumbent Low Flank Approach

With the cow in left lateral recumbency, the incision is made from approximately 15 cm below the transverse processes of the lumbar vertebrae, extending down to just above the milk vein (see Fig. 16.17). The three abdominal muscles are sharply incised in the same direction. After the abdominal cavity is opened, the cow is pushed up into a near sternal position. Now, the pregnant horn is rolled out of the abdomen on to a large plastic sheet or apron, which had been sterilized or disinfected beforehand. The uterus is incised, its contents removed and the incision closed, using a double suture line. The flank incision is then closed as described for the left paralumbar fossa approach. Although this particular approach has not been tried and tested, it may be a useful alternative technique in the field for the surgical delivery of a grossly emphysematous calf.

Intraoperative Management

The emphysematous fetus presents an unavoidable risk of peritoneal contamination, not least because hair and hooves may already have been shed. In such cases, incision of the uterus is often followed immediately by the escape of gas and fetid fluid; parts of the fetus may be grossly swollen, dry, and crepitating on handling. The uterine wall is often tightly stretched, and intrauterine manipulation can be very demanding. Therefore it is essential to make the abdominal wall and uterine incisions of adequate length. Such a fetus often requires considerable traction, not only when using limb chains but also with sharp or blunt hooks applied in the orbits and at appropriate points on the trunk or upper limbs to secure additional purchase. Before

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removal is possible, it may be necessary to make several deep incisions at different sites over the fetal thorax and abdomen in order to release gas and, sometimes, to partially eviscerate the fetus. Incising the fetal abdominal wall may also be necessary in case of ascites. Sporadically, the fetus simply cannot be removed from the uterus because it is impossible to make a uterine incision of adequate length. In such cases a partial fetotomy through the laparotomy site may be attempted.

Success Rates and Complications of Surgery

Fetal survival after delivery by Caesarean surgery partly depends on the indication for surgery. However, Barkema et al. (1992) reported a calf mortality rate of 12% after Caesarean hysterotomy, compared with 5% for control calvings. In the study of Dobranic et al. (2001), the proportion of calves that were born alive was greater if the duration of dystocia was less than 12 hours.

Maternal survival rates after Caesarean hysterotomy are usually high; most surveys report 90% to 98% dam survival (Dehghani & Ferguson 1982, Cattel & Dobson 1990, Dawson & Murray 1992). Sellart and Campero (2008) reported a maternal survival rate of 97.4%. In a series of 1134 surgeries performed primarily for dystocia, Pearson (1996) reported an 88% maternal survival rate despite the fact that 37% of calves were dead at the time of surgery.

Cows that have emergency Caesarean hysterotomy are more likely to experience intraoperative and postoperative complications and are less likely to survive than cows that had an elective Caesarean (Newman & Anderson 2005). Thus, Bochard et al. (1994) found that the survival of the dam was influenced by the status of the calf: risk of maternal death was 63%, 21%, and 14%, respectively, if the calf was emphysematous, dead, or live. However, Vandeplassche (1963) reported that 80% of cows survived even when an emphysematous fetus was present. A more recent study of 103 cases of Caesarean hysterotomy performed in one predominantly dairy practice found that the 14-day cow survival rate was 80.6%, and of those surviving beyond this period, 55.4% carried another calf to term, 27.7% were culled due to infertility, and 16.9% were culled for other reasons (Lyons et al. 2013).

Complications associated with Caesarean hysterotomy are often due to difficulties encountered during rotation of the uterus, exteriorization of the pregnant horn, making the uterine incision, removing the calf, and suturing the uterine incision. Exteriorizing the uterus is the most common difficulty experienced during Caesarean hysterotomy: Hoeben et al. (1997) found that the uterus was difficult to exteriorize in 21% of surgeries and impossible to exteriorize in 6%. They also noted that the ability to exteriorize the uterus was associated with the experience of the surgeon. Failure to exteriorize the uterus is associated with reduced survival of the cow, especially if the fetus is dead.

Complications that have been reported to occur after Caesarean hysterotomy are:

Subcutaneous Emphysema

If the edges of the peritoneal incision are not fully apposed, air often leaks from the abdominal cavity into the subcutaneous tissues and muscle layers after surgery, causing emphysema (Sloss & Dufty 1977). In some cases it may extend as far as the shoulders. The condition is more common in animals that continue to strain after surgery, usually as a consequence of the dystocia. Although unsightly, it has no significant detrimental effect on the animal and treatment is not required. Usually, the gross appearance of the animal will return to normal in 1 to 8 weeks, depending on the amount of air that leaked into the tissues.

Metritis and Retained Fetal Membranes

Retention of the placenta occurs in 6% to 10% of cases of Caesarean hysterotomy. Dystocia, twins, uterine torsion, and fetal monsters are common indications for Caesarean hysterotomy; the procedure itself predisposes to retained fetal membranes. Removal of the membranes during surgery is rarely possible, but the placenta is normally expelled within 4 to 6 hours after surgery. However, if the membranes are still retained more than 24 hours after surgery, gentle attempts at removal can be carried out daily by exploration of the vagina only. The risk of retention of the placenta may be reduced by administering $PGF_{2\alpha}$ after removal of the calf (Stocker & Waelchli 1993). The intramuscular administration of antibiotics can be considered, although most literature recommends no treatment in uncomplicated cases. Once the membranes have been expelled, usually as a necrotic mass after approximately 7 to 11 days, gentle lavage of the uterine lumen with 5 litres of warm, normal saline can be carried out, using a sterile, wide-bore tube.

Adhesions

To date, no clinical research has documented any beneficial effect of prophylactic treatment, such as lavage of the uterus, NSAIDs, and intraabdominal antibiotics, to prevent adhesion formation in cattle. Still, it is not uncommon for adhesions to develop after Caesarean hysterotomy. Neither the suture pattern (i.e., Utrecht method, Lembert, or Cushing) nor the suture material (i.e., plain and chromic catgut, polyglycolic acid, and polyglactin 910) used to repair the uterine incision appears to be specifically important (Anderson 1998). However, the actual surgeon seems to have the greatest influence on the number and severity of adhesions that occur. The surgeon's speed, skill, and dedication are mentioned in the literature as being the best prevention against adhesion formation. Also, it is vital to obtain a good seal of the uterine incision, whenever possible, and to avoid excessive tissue handling, trauma, ischaemia, and haemorrhage.

The use of synthetic absorbable suture material, such as polyglactin 910 and polyglycolic acid, does not seem to offer any real advantage as far as the development of adhesions is concerned. However, from a surgical point of view, these materials have excellent handling qualities, are more pliable than catgut, hold knots well, and do not fray. They also have a higher guarantee of uniform material quality compared with a biological product such as catgut. Besides the high cost of synthetic suture materials, there is another important disadvantage. Their braided structure causes more drag as they pass through tissue, which may result in small tears, especially when the uterine wall is oedematous. Catgut, on the other hand, is more easily damaged by contact with the needle. This can cause the uterine suture to break, which would inevitably result in a severe peritonitis.

Nowadays, the trend among cattle surgeons is to use large-diameter (6 USP or 10 metric), plain catgut for closing the uterine incision. Although this material is more reactive than chromic catgut or the synthetic materials, it may actually result in fewer adhesions, possibly because of the associated time factor. Because of the abundant blood supply in the rapidly involuting uterus, the suture material is probably absorbed so quickly that adhesions have less chance to form compared with suture materials that remain *in situ* for longer periods of time. The knots are the last portion of the suture line to be absorbed, probably because

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cellular invasion is more difficult. Therefore burying the knots in folds of uterine tissue at both ends of the incision, as is achieved when using the Utrecht method, should always be the primary goal when suturing the uterus.

Peritonitis

Diarrhoea, pyrexia, inappetence, and abdominal pain are the common presenting signs of peritonitis after Caesarean hysterotomy. Fortunately, the omentum and/or the use of antimicrobial therapy often limit the spread of peritonitis within the abdominal cavity. In many instances, however, there are recurrent cycles of peritonitis and healing, leading to formation of extensive adhesions and chronic weight loss.

Inadequate repair of the uterine incision, particularly in the presence of a metritis, is the principal cause of postoperative peritonitis. However, in some cases, the peritonitis may already exist at the time of surgery. The incidence is increased in case of a dead or emphysematous fetus, after severe dystocia, rupture of the uterus, or presence of a fetal monster, and after spillage of infected uterine fluids into the abdomen during surgery. However, spilling small quantities of uterine fluid into the abdominal cavity during calf removal appears to be of little consequence to the outcome of the Caesarean hysterotomy no matter whether the fetus is alive or dead, but still fresh.

A variety of treatments have been suggested in case of peritonitis, including parenteral antibiosis, intraabdominal administration of antibiotics through the right flank, surgical lavage of the peritoneal cavity, and intravenous fluid therapy.

Wound Dehiscence

As many as 6% of animals may have complications related to dehiscence, abscess, or seroma formation around the abdominal incision (Dehghani & Ferguson 1982). Predisposing factors for wound dehiscence include inadequate asepsis, low abdominal incisions, overzealous handling of tissues during surgery, environmental contamination, tenesmus, and poor temperament of the animal after surgery. In addition, removal of skin sutures too early after surgery can lead to the incision line opening up; 3 weeks is a minimum period before removing sutures.

Serum-like fluid occasionally accumulates at the ventral aspect of the wound between the muscle layers if the dead space is not fully occluded; it usually resolves spontaneously or can be drained surgically.

In other cases, there may be formation of an abscess. In most instances, the abscess can be lanced, drained, and irrigated as a granulating wound, followed by second intention healing. Antibiotic treatment is usually not required but may be necessary if there is pyrexia.

Nerve Paralysis

Cows that are recumbent during surgery have the risk of temporary or permanent peroneal nerve injury. Animals that have been in lateral recumbency on a hard surface may suffer from radial paralysis. In addition, a number of cows may have sustained trauma to the obturator nerve as a consequence of dystocia before surgery. More often, however, femoral paralysis is encountered as a 'stretch injury' in large calves, mainly of beef breeds, after hip- or stifle-lock during parturition.

Fractures

The dam may sustain a fracture when attempting to rise after surgery. However, more common is for the calf to sustain a long bone fracture(s) or growth plate separation during attempts at correcting dystocia before surgery.

Postpartum Haemorrhage

Haemorrhage from the abdominal incision is usually limited, although dependent on the haemostatic concern of the surgeon. However, haemorrhage from the uterine incision can be considerable and in some cases fatal, especially when cotyledonary vessels are disrupted. Occasionally, the haemorrhage may be minimal during the surgical procedure, but may progress in the 24 hours after surgery. Furthermore, in sporadic cases the large vessels present in the broad ligaments may be damaged, causing considerable blood loss. Prevention of these causes of haemorrhage is by careful incision of the uterus (avoiding cotyledons), adequately supporting the uterus during surgery (especially when suturing the uterine incision), and close attention to haemostasis. Treatment of severe, life-threatening haemorrhage is by blood transfusion. In addition, 20 to 40 IU oxytocin may be administered repeatedly to stimulate uterine contractions in an attempt to reduce uterine haemorrhage.

Blackleg

Dehghani and Ferguson (1982) reported that 0.5% of cases died suddenly within 24 hours of surgery as a result of blackleg, with the muscle lesions located some distance from the surgery site.

Postoperative Fertility

Under normal circumstances the three main goals of the Caesarean hysterotomy in cattle are survival of the cow, survival of the calf, and maintenance of postoperative productivity, implying not only the maintenance of body condition and an acceptable level of lactation but also the ability to conceive again and sustain a developing fetus to term. Numerous data have been published on fertility rates after Caesarean hysterotomy, but their significance is qualified by the fact that many animals were culled without being inseminated or served again. In 10 such series, the percentage of cows that subsequently conceived postoperatively ranged from 48% to 80%, with a mean value of 72% for 2368 animals, compared with 89% for normal calvings (data cited by Boucoumont et al. 1978). Vandeplassche et al. (1968) reported that 60% of 1857 cows and heifers that had had Caesarean surgery were subsequently inseminated and 74% of these eventually conceived with an average of 1.8 inseminations per conception. However, there was an increased incidence of abortion, hydrallantois, and failure of the cervix to dilate at the next parturition, probably due to scar tissue in the uterine wall. Although the calving interval is increased in cows after Caesarean hysterotomy compared with normal calvings, the principal cause of economic loss is the higher culling rates (Barkema et al. 1992). Interestingly, in the latter study the calving-to-first-insemination interval was similar between Caesarean cows and control cows, but the calving-to-conception interval was 18 days longer. Lyons et al. (2013) reported a 14–day cow survival rate after Caesarean hysterotomy of 80.6%; of these, 27.7% were subsequently culled due to infertility. Reduced fertility may result as a consequence of increased incidence of retained fetal membranes and endometritis, uterine adhesions that hinder involution, adhesions that affect the ovary or uterine tube, and reduced competence of the endometrial tissue. In addition, there is an increased frequency of abortions during subsequent pregnancies, possibly as a result of scar tissue formation within the uterine wall, restricting the expansion of the uterus and/or nutrition of the fetus.

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Sheep, Goats, and Deer

Indications

The main indications for Caesarean surgery in the ewe, doe, and hind are similar to those in other species:

- Fetopelvic disproportion, particularly in primiparous animals with a single fetus
- Failure of the cervix to dilate (so-called 'ringwomb')
- Irreducible or severely traumatised vaginal prolapse
- Fetal emphysema after protracted dystocia

Less frequent indications are uterine torsion, vulvovestibular stricture, and faulty fetal disposition that cannot be corrected because of maternal immaturity or uterine contraction. Occasionally, a Caesarean hysterotomy is done to terminate pregnancy when the ewe suffers from pregnancy toxaemia. Vaginal prolapse should initially be treated conservatively by repositioning and inserting vulvar retention sutures in the hope that pregnancy will continue to term (see Chapter 10). However, many cases undergo early labour with incomplete dilation of the cervix. Unfortunately, lambs from such animals frequently die of prematurity, after showing characteristic convulsive limb movements and respiratory embarrassment. The success of Caesarean surgery in sheep is largely dependent on the early recognition of dystocia by the farmer. Sadly but not surprisingly, considering the economic value of most commercial ewes, the vast majority of Caesarean operations are carried out as a last resort. In the study by Brounts et al. (2004), over half (116 of the 201) lambs and kids delivered were dead or died shortly afterwards; this can be explained by the fact that 42 of the 65 dams had been in the second stage of parturition for more than 6 hours. In the authors' experience, however, ewe and lamb survival rates are high when surgery to relieve the dystocia is carried out promptly.

A common indication for Caesarean hysterotomy in deer is faulty fetal disposition that cannot be corrected by other means (e.g., lateral or ventral flexion of the head). Although fetotomy would be the preferred method to relieve this type of dystocia, this is not always possible.

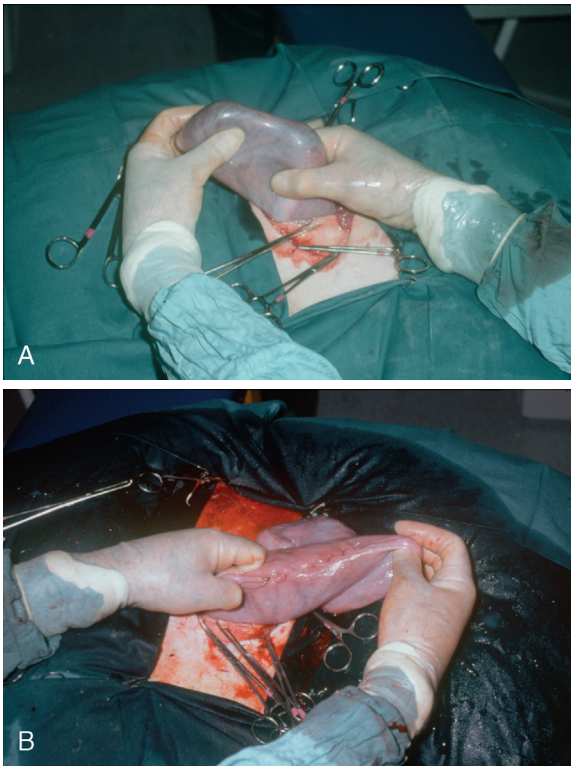
Anaesthesia

Hysterotomy is usually performed through a left flank incision under paravertebral, inverted-L nerve block or local infiltration analgesia with the animal positioned in right lateral recumbency, using 2% lignocaine. Caution is essential when inducing local analgesia in sheep because accidental intravenous administration or the injection of an excessive amount of lignocaine may cause convulsions. A lumbosacral epidural will also provide good anaesthesia, but one should be watchful for possible shock.

Operative Technique

The most commonly used surgical techniques are right lateral recumbency with a paralumbar incision, dorsal recumbency with a ventral midline or paramedian incision, and dorsal recumbency with a ventral abdominal incision located in the naturally denuded area above the udder. The first listed technique is the authors' preferred approach and is described later in this section. Using this approach, there is no depression of the fetus(es), and the ewe experiences less respiratory embarrassment than when positioned in dorsal recumbency. Some surgeons, however, favour the ventral abdominal approach because less wool needs to be removed from the surgical field (Ames 2014). In deer the authors' preference is for the right lateral recumbency approach with a mid paralumbar incision.

The ewe is positioned in right lateral recumbency with rope restraint. In case additional restraint is still needed, sedation with xylazine or diazepam can be used. The left paralumbar region is close-clipped and the skin prepared for aseptic surgery. In deer, it is best to remove the coarse hair with a pair of large animal clippers, as close shaving is difficult. The surgery site should be covered with a large, fenestrated, moisture-repellent drape. A vertical incision is made in the midparalumbar fossa, and the underlying muscles are incised in the same way as described previously for the cow. However, compared with bovine tissues, ovine tissues are more fragile and thin; they should be handled with care. In this context it is important to stress that the body wall is much thinner than in the cow, and thus care must be taken not to incise the rumen accidentally. With a high paralumbar incision it is also essential to identify the highly vascular mesometrial attachment to the parietal peritoneum. After brief exploration of the abdominal cavity to determine the number of lambs, a gravid horn is partly lifted through the abdominal incision. A fetal extremity, preferably the hock, is grasped through the uterine wall so that an incision can be made in the same way as that described for the cow (Fig. 16.18A). However, it is important to stress that more than a single fetus is likely to be the norm, potentially making identification of which extremity belongs to which fetus difficult. It is even more important to remember to explore the entire uterus, particularly the horn opposite to the one incised, to ensure that all lambs have been removed before suturing the uterine incision. Usually, it is possible to remove all lambs through a single uterine incision by manoeuvring the fetus(es) situated in the opposite horn around the uterine bifurcation.



• **Fig. 16.18** Caesarean hysterotomy in the ewe. (A) The exteriorized uterus over a fetal forelimb. (B) Hysterotomy repair with inversion sutures.

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The fetal membranes should be removed if they can be readily detached; if not, then the part(s) that cannot be returned to the uterine lumen, thus interfering with the closure of the uterine incision, should be trimmed off. The uterine incision is closed with a single, continuous inversion suture pattern, such as Utrecht's, Lembert's, or Cushing's (see Fig. 16.18B), using absorbable suture material, such as 0 USP (3.5 metric) or 1 to 0 USP (4 metric) plain or chromic catgut or a comparable synthetic suture, swaged on an atraumatic, round-bodied needle. After prolonged dystocia, oxytocin (3–5 IU) can be administered after uterine closure to promote contraction of the uterus and milk letdown. Each layer of the abdominal wall should be sutured in a continuous pattern with absorbable suture material. Skin closure is with a continuous interlocking or interrupted pattern using nonabsorbable suture material. Systemic antibiotics should be given as deemed prudent. Sheep, more than any other species, are highly susceptible to the toxaemic effects of intrauterine clostridial infection, and most deaths are due to this complication.

The techniques and approaches for Caesarean hysterotomy in goats and deer are comparable to those used in sheep.

Postoperative Fertility

Only scant information exists on the fertility of ewes and doe goats after Caesarean hysterotomy, because often ewes, and to a lesser extent does, will be culled either because they had the procedure done or because of the nature of the dystocia. Brounts et al. (2004) found that of the 16 ewes and does that were rebred (admittedly a very small percentage of the 110 that had Caesarean hysterotomy to relieve dystocia) all conceived, and none suffered subsequently from dystocia. This confirms the authors' experience with experimental ewes that fertility is not impaired to any extent, probably because of the seasonal pattern of reproduction with a long anoestrous period, allowing for adequate healing of the genital tract.

Horse

Casearean hysterotomy in the mare is still widely regarded as a serious and difficult procedure. Even so, the mare tolerates this surgical interference as well as most other species, and the recovery rates are generally good. However, there is little doubt that improved methods of general anaesthesia and postoperative care have greatly improved the chances of maternal recovery. Even in specialist equine hospitals in areas of high stud density, Caesarean hysterotomy is not a common procedure. Perhaps a reflection of overall improved anaesthetic and surgical techniques can be gleaned by comparing the observations of Vandeplassche et al. (1977), who commented that they performed 15 fetotomy procedures for every Caesarean, with those of Freeman et al. (1999a). In the latter study, performed at two university veterinary hospitals in the United States 22 years later, there were 66 Caesarean operations and 50 vaginal deliveries. This study indicated that Caesarean hysterotomy was preferable to delivery of the foal by controlled vaginal delivery under general anaesthesia in situations in which correction of the dystocia was difficult and prolonged.

If the foal is alive, the surgery should be carried out with minimum delay. If the fetus lies already in the maternal pelvic canal, it suffers fatal anoxia due to dehiscence of the allantochorion within 1 to 2 hours of the beginning of second stage labour. This observation is corroborated by the fact that 70% of foals born by hysterotomy at the University of Ghent were stillborn or died soon after delivery (Vandeplassche 1980). Resolute surgery in the field may therefore be more beneficial than referral to a specialist hospital setting. One particular complication of Caesarean hysterotomy in the mare is rupture of the uterine artery during manipulation of the gravid uterus (Threlfall 1997).

Indications

The range of indications is more limited than that in cattle. Cervical dystocia is not recognized in the mare, and fetomaternal disproportion and fetal monsters are less common than in other species. The major indication in the Ghent series, accounting for 39 of 71 cases, was bicornual pregnancy or transverse presentation, followed by other faulty fetal dispositions, complicated by injury, contraction, or infection (13 cases), and uterine torsion (10 cases). In a much smaller series of 34 cases at the veterinary school of the University of Bristol, uterine torsion was the most common indication.

With their considerable experience of equine dystocia, Vandeplassche et al. (1977) regard the following indications as absolute:

- Faulty fetal disposition that cannot be corrected by other means (e.g., transverse presentation)
- Vulvovaginal or uterine trauma
- Vaginal oedema
- Uterine torsion that is not correctable by a vaginal approach or by rolling the mare
- Severe congenital deformities (e.g., 'wryneck', ankylosed limbs, hydrocephalus)

In these forms of dystocia, Caesarean hysterotomy should be the primary method of delivery rather than a last resort.

Significantly, these authors also specify forms of dystocia that they consider as contraindications for surgery; these include lateral deviation of the neck, breech presentation of a dead fetus, twin dystocia, and prolapse of the maternal bladder.

Anaesthesia

Correction of preparturient uterine torsions can be carried out by laparotomy in the standing animal, using local analgesia or nerve block (Vandeplassche 1980). New and improved anaesthetic agents and better patient monitoring have significantly reduced the risk to the mare during Caesarean surgery performed at term. For details of anaesthesia in this species the reader is advised to consult a specialist textbook. It is important to stress that there may well be a conflict between the obstetrician and the anaesthetist because dorsal recumbency may induce 'supine hypotension' if the gravid uterus compresses the posterior vena cava, thus impeding venous return and reducing cardiac output. For this reason the mare should be placed in lateral or dorsolateral recumbency during preoperative preparation and retained in dorsal recumbency for as short a period of time as is appropriate for effectively carrying out the actual surgery.

Operative Technique

Potential surgical sites in the standing animal include right or left flank, using sedation and local anaesthesia. However, this approach is seldom used because of reduced exposure of the uterus, visible abdominal wall scarring, and the risk of animal movement during surgery (Threlfall 1997). The same flank approaches using general anaesthesia are also options, although some of the previously mentioned disadvantages (i.e., limited exposure and scarring) still remain. Possible surgical approaches in a recumbent animal include low incisions through either of the ventral flanks, the paramedian,

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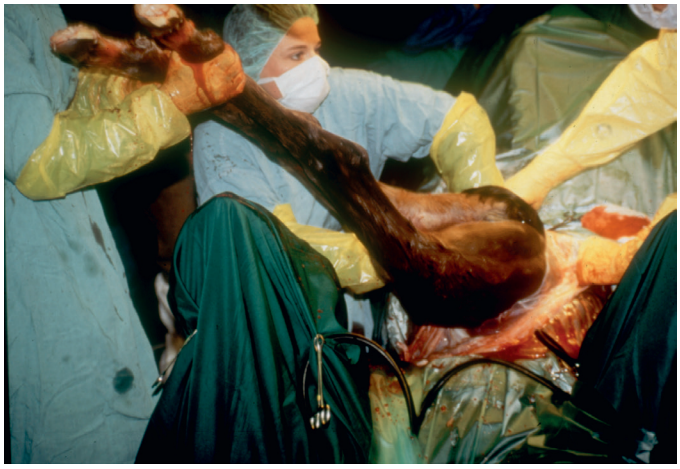
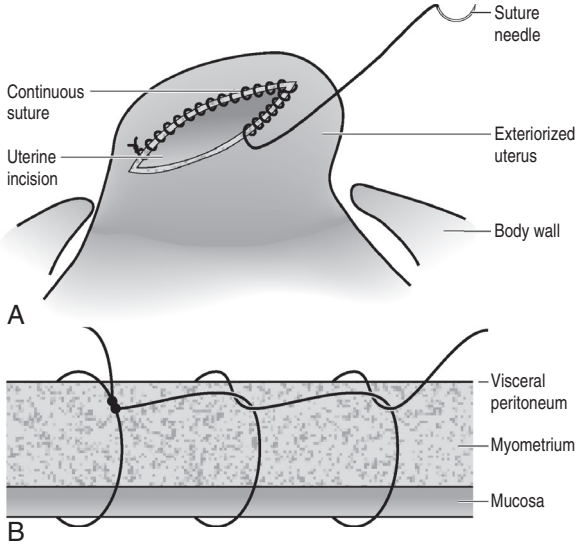


• **Fig. 16.19** The foal is removed from the uterus by the surgeon and assistant pulling on the hindlimbs.

• **Fig. 16.20** Physical separation of the allantochorion from the endometrium for several centimetres from the edges of the uterine incision.



• **Fig. 16.21** The use of a continuous lockstitch suture through the whole thickness of the uterine wall, from serosa to endometrial surface, as described by Vanderplassche (1973).

or ventral midline. The ventral midline incision is now widely adopted for gastrointestinal surgery. It is even more satisfactory for Caesarean hysterotomy because it provides excellent exposure of the uterus and considerably reduces intraabdominal pressure so the wound can be repaired easily without excessive tension on the suture lines. In addition, there is very little haemorrhage when this site is used. Sometimes, one or two large veins exist in this area, creating an anastomosis from the right to left subcutaneous abdominal veins (Threlfall 1997). If so, these vessels should be ligated before proceeding with the incision in the subcutaneous tissues. All other approaches necessitate muscle division, which results in greater operative haemorrhage and postoperative oedema. Provided that the midline incision is properly repaired, the risk of incisional hernia is negligible.

The mare's uterus is seldom so tightly contracted that a fetal limb cannot be grasped through the uterine wall and brought through the abdominal incision. For this reason a uterine incision of adequate length is easily made on the greater curvature of the gravid horn with little risk of tearing during manipulation of the fetus. The fetus is then extracted, making maximum use of joint flexibility, and gently supported outside the abdomen with its umbilical cord still intact (Fig. 16.19). The equine fetus is significantly less sensitive to 'pinching' stimuli *in utero* than the bovine fetus; therefore, fetal viability should be assumed until cord or heart palpation proves otherwise, unless the placenta has fully separated. If the foal is alive, the cord is left intact for several minutes until breathing begins. The cord is then ligated or preferably divided by stretching. If the foal is dead, the placenta may already have separated. It can then be easily removed through the uterine incision. In many cases, hysterotomy is followed immediately by profuse haemorrhage from the subendometrial plexus of arteries and veins, these being too numerous to ligate individually. As a means of controlling such haemorrhage, Vandeplassche (1973) recommended the insertion of a continuous suture through all layers of uterine wall along the edges of the uterine incision, immediately after the placenta has first been detached from the area near the incision (Figs 16.20 and 16.21). However, its value has been questioned and, in a study involving 66 mares in which the suture was used in 31 and not used in 35, the length of the surgical procedure was significantly shorter ($p < 0.05$) in the latter group, and there was no difference between the two groups for the numbers of mares that had anaemia after the surgery (Freeman et al. 1999b). Nevertheless, anaemia was found to be five times more common after Caesarean hysterotomy than after vaginal delivery. Therefore it is recommended that, if the suture advocated by Vandeplassche (1973) is not used, a full thickness simple continuous, continuous interlocking, or similar suture pattern is used that is sufficiently tight to compress the vessels in the uterine wall.

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If the placenta remains attached to the endometrium, it is better not to attempt manual separation because this procedure not only results in diffuse endometrial bleeding but also in the retention of microvilli, which predisposes to subsequent endometritis. The uterine incision is repaired using polyglycolic acid suture material in a Cushing pattern to invert the incision site. One or two rows are used, depending on whether the first row of suturing tears through the uterine wall, which is sometimes noticeably fragile. After the removal of clotted blood and other debris, a soluble antibiotic preparation may be sprinkled on the uterine incision.

The abdominal incision is easy to suture because of flaccidity of the stretched abdominal musculature. It is important to insert closely spaced sutures of appropriate material in a continuous or interrupted pattern. There is no need to stitch the peritoneum and layer of subperitoneal fat. The laparotomy repair is completed with a continuous subcutaneous suture and appropriate stitches in the skin.

Postoperative Management

After Caesarean hysterotomy, oxytocin should be administered to induce uterine contraction even if the placenta has been removed at surgery. In the study by Freeman et al. (1999a) the fetal membranes were retained in 65% of the mares, which included 22 out of a total of 116 that had a vaginal delivery to correct the dystocia; it was retained much longer in those mares that had elective surgery. Vandeplassche et al. (1971) recommend immediate oxytocin therapy followed by a supplementary slow intravenous infusion of 50 IU in saline if the placenta is not expelled within 4 hours. The latter method of administration probably has a more physiological effect. Experience suggests that oxytocin therapy in the mare is sometimes followed by excessively vigorous uterine contraction and eversion of the uterine horn into the vagina, threatening eversion through the vulva, even after the placenta has been expelled. Nevertheless, repeated administration of oxytocin (e.g., every 4 hours) is advisable to enhance uterine involution (Threlfall 1997). After oxytocin therapy, the placenta is usually expelled within 12 hours. In occasional cases it may separate but remain within the uterus and anterior vagina and is then easily removed through vaginal exploration. Nowadays, retention for longer than 24 hours is not regarded as an indication for immediate manual separation, provided that antibiotic therapy is maintained. However, removal may still be justified in draught-type mares, which are particularly susceptible to systemic reactions. Although intrauterine antibiotic administration may have a beneficial effect after removal of the placenta, more important by far is siphoning of any uterine fluid that accumulates, especially in mares that show signs of anaphylaxis or are not recovering satisfactorily. Vandeplassche et al. (1977) have commented on the 2- to 3-day delay in uterine contraction after Caesarean hysterotomy and recommend the breaking up of perimetrial adhesions per rectum at the end of this period.

In general, antibiotic therapy is provided pre- and postoperatively, especially if the foal has been dead for some time, which may have resulted in putrefaction.

Abdominal incisions in the horse are usually followed by local oedema of varying severity. After midline laparohysterotomy, diffuse subcutaneous oedema may extend along the ventral abdomen as far as forward as the presternal region; however, the swelling usually subsides slowly over a period of 7 to 10 days. The administration of diuretic agents appears to make the oedema disappear more quickly, but the necessity for such treatment is questionable. Wound infection is treated by removal of appropriate skin sutures to provide drainage.

Foal Survival, Maternal Recovery Rate, and Causes of Death

As has been mentioned previously, placental separation occurs rapidly in the mare; hence the delivery of a live foal from a Caesarean hysterotomy is uncommon unless it is elective surgery. Six out of 19 foals (Juzwiak et al. 1990) and 11 out of 102 foals were alive at the time of surgery, of which only five were alive at the time of discharge from hospital (Freeman et al. 1999a). Foal survival was much better after an elective Caesarean hysterotomy with a survival rate of 90%, although it fell to 38% if done concurrently with surgery to treat colic (Freeman et al. 1999a).

If the dystocia is of short duration, the prognosis for maternal recovery is good. In the Ghent series of 77 surgeries, 62 mares (81%) recovered (Vandeplassche et al. 1977). The Ghent data suggest that most deaths occur during or very soon after surgery and are attributable to shock caused by uterine haemorrhage or gross uterine infection. Haemorrhage can be largely prevented by haemostatic suturing of the uterine incision. The systemic effects of fetal emphysema and other forms of shock can be countered by intensive, intravenous fluid therapy during and after the surgery. Juzwiak et al. (1990) reported that 17 out of 19 mares survived postoperatively until the time of discharge, the most common complications being abdominal pain (13 mares), anaemia (10 mares), and retained fetal membranes (6 mares).

Because most deaths occur during the immediate postoperative period, the clinician is more likely to be worried by two particular complications that may develop within a few days after surgery. The onset of diarrhoea should be viewed with the greatest concern because body fluid loss is rapid and severe, and fluid reserves are soon depleted even if the mare continues to drink. The role of antibiotics in the pathogenesis of this disorder and their value in its treatment are unclear, but there is no argument about the necessity for immediate fluid replacement therapy to maintain hydration and normal electrolyte status. The other complication is laminitis, which has long been recognized as a sequel of placental retention in animals of the heavy draught breeds. The earliest sign of this supposedly anaphylactic reaction may be severe pulmonary oedema with dyspnoea and nasal regurgitation of fluids. Pedal pain is then manifested by reluctance to move or even stand, and without careful clinical examination the resultant recumbency during the early postoperative period may easily be mistaken for terminal illness justifying euthanasia. In such cases, accumulated uterine fluids should be removed immediately by careful siphoning. The use of diuretics is indicated for remedying severe oedema, whereas laminitis should be managed by dietary restriction and pain control.

Postoperative Fertility

Juzwiak et al. (1990) reported that 50% of mares bred after Caesarean surgery produced at least one other foal. Stashak and Vandeplassche (1993) found that, in a series involving 82 mares that had had Caesarean surgery, 34 (41.5%) were not bred again; of the 48 (58.5%) that were bred, 28 (58.3%) became pregnant. In the same study, of the 28 mares that became pregnant, 23 (82.1%) foaled at term; of those that did not, most aborted at various stages of gestation. Thus Caesarean surgery in the mare should not have too great an effect on subsequent fertility, provided it is performed

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quickly after the onset of dystocia and before there is heavy bacterial contamination of the uterus either from manual interference or from putrefaction of the foal. The report by Arthur (1975) of two mares that each conceived after two elective surgeries suggests that the hysterotomy per se is less important in this respect than the state of the fetus and the uterus at the time of surgery.

Pig

The sow, similar to the bitch, is a difficult obstetrical patient because it is not always possible to identify a particular cause of dystocia even after Caesarean surgery has been carried out, although the need for surgery may be quite clear. Under field conditions, Caesarean surgery is usually attempted after the sow has been in labour for at least 6 to 12 hours (Noordsy 1994). Generally, the prognosis for the dam and piglets becomes guarded to unfavourable in dystocias of 12 hours or more.

Indications

In a series of 57 surgeries reported by Renard et al. (1980), the major indications were: irreducible vaginal prolapse (32%); fetopelvic disproportion, including fetal emphysema (32%); secondary uterine inertia (23%); and, surprisingly, nondilation of the cervix (10%). Preparturient vaginal prolapse may be complicated by rectal prolapse and retroversion of the urinary bladder, and even of the gravid uterus. Such a prolapse has often undergone considerable trauma and marked oedematous swelling. A fresh prolapse at term does not necessarily interfere with parturition. However, in cases in which manual delivery is required, oedema rapidly develops and tissues tear readily. Uterine inertia of a primary or secondary nature is an important indication for surgery, and because of delay, the fetuses in such cases are often emphysematous. Primary inertia is frequently associated with 'deep-sided' sows and a hyperthermic environment (Noordsy 1994). Especially in cases of secondary inertia, it is not always easy to be certain that fetuses are still retained in the uterus. If they cannot be palpated or balloted through the uterine or abdominal wall and if fetal heart sounds cannot be detected, radiography or ultrasonography is advisable before surgery is undertaken. Less frequent clinical indications for Caesarean hysterotomy are maternal immaturity and pelvic deformity, torsion of one or both uterine horns, lacerations of the birth canal due to improper manual attempts to alleviate dystocia, and fetal deformities such as hydrocephalus or conjoined piglets. Preparturient elective hysterotomy is also performed as an alternative to gravid hysterectomy to obtain pathogen-free piglets, which are then fostered or reared artificially.

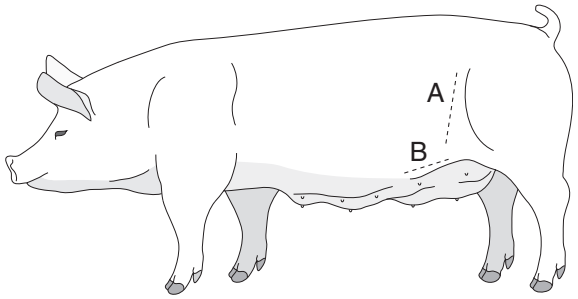
Anaesthesia

Because of difficulties in restraint, the surgery is generally performed using deep sedation and local analgesia or general anaesthesia. The best method under field conditions is a combination of azaperone (1.0 mg/kg) and ketamine (2.5 mg/kg) intramuscularly as a sedative, followed by intravenous ketamine (2 mg/kg) and midazolam (100 µg/kg) about 15 minutes later (Clutton et al. 1997). Once adequately anaesthetized, the sow can be intubated and the general anaesthesia maintained with oxygen/nitrous oxide/halothane. Alternatively, it is possible to perform the Caesarean surgery using local infiltration and the ability to 'top up' with further doses of the latter combination intravenously. Brodbelt and Taylor (1999) reported the use of two combinations of drugs injected intramuscularly, which is a much easier technique than the intravenous route. The combinations were azaperone (2 mg/kg), butorphanol (0.2 mg/kg), and ketamine (5 mg/kg) or detomidine (100 µg/kg), butorphanol (0.2 mg/kg), and ketamine (5 mg/kg). Although both combinations allowed endotracheal intubation, it is likely that Caesarean hysterotomy can be carried out with the use of local infiltration at the surgery site, i.e., without inhalation anaesthesia.

Renard et al. (1980) recommended the use of anterior (lumbosacral) epidural analgesia; however, they also reported a very high incidence of postoperative hindlimb paresis, which was attributed to lateral recumbency on a hard surface. Provided that the animal is adequately restrained under sedation, local analgesia or a paravertebral nerve block can also be successfully used, especially in toxic sows. However, it is important to avoid unnecessary struggle with hypotensive surgery subjects.

Operative Technique

Several different methods of restraint and approaches have been described, including right- or left-sided lateral recumbency restraint with either a vertical midparalumbar or horizontal low ventral incision (10–15 cm dorsal and parallel to the thickly developed mammary tissue), and dorsal recumbency restraint with a ventral midline incision (Noordsy 1994). A vertical paralumbar fossa or ventral flank incision on either side is the preferred site (Fig. 16.22). The choice between right or left side is that of the surgeon and is usually dictated by his/her dexterity. Anatomically, there is no obvious advantage for either side. The incision site should be clipped using a No 10 head set of small animal clippers and scrubbed for aseptical surgery. An adequate-sized, fenestrated, moisture-repellent drape is applied to the surgical area. A vertical incision is made in the midparalumbar region, large enough to allow easy insertion of the surgeon's arm. After cutting or spreading the muscle layers, the peritoneum will often bulge outward when exposed. It is grasped with forceps, punctured and then incised carefully because of its usually close contact with the underlying abdominal viscera (Noordsy 1994). Each gravid horn should be exteriorized in turn for incision outside the peritoneal cavity in order to minimize peritoneal contamination. A fetus is grasped within one of the uterine horns and brought through the abdominal incision (still enclosed in the uterus). The incision should be made longitudinally on the greater curvature of the uterine horn. If the fetuses are not emphysematous, it is usually possible to evacuate both horns through a single incision in the centre of each horn, with the piglets at the ovarian poles and the base of the uterine horns being squeezed down the horn and grasped through the incision. In cases in which the fetuses are emphysematous, multiple



• **Fig. 16.22** Position of skin incision for Caesarean surgery in the sow: (A) in the left paralumbar fossa or (B) ventral flank.

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incisions sited directly over or between them may be necessary. It is important to keep the uterine tissue moist during the surgery using a normal saline solution, with or without soluble antibiotics. The piglet's umbilical cord is long and, even without placental separation, forceps clamping or ligation is usually possible before division. Fetal membranes that have not separated should be left *in situ* and not forcibly removed by traction. Because the uterine horns are long, it is important to palpate the genital tract in its entirety to ensure that all piglets have been removed. The uterine incisions are repaired with inversion sutures, using 2 to 0 USP (3 metric) medium chromic catgut or a comparably sized synthetic, absorbable suture material, and a swaged, atraumatic needle. The sow's uterus, similar to the mare's, is apt to tear if the suture is pulled too tight, but this is of little, or no consequence if rapid contraction is induced by postoperative oxytocin therapy. Finally, the uterine horns are placed in the abdominal cavity in their normal position, making sure they are not accidentally crossed. Before closing the abdominal incision, Noordsy (1994) advises to administer intraperitoneal soluble tetracycline. The peritoneum and muscle layers are sutured separately, using a simple continuous suture pattern. Moderate tension should be placed on the muscle suture lines. The skin is closed with extra heavy, synthetic, nonabsorbable suture material (e.g., Supramid Extra), using a large, curved cutting-edge needle capable of easily going through tough pigskin without bending. An interrupted cruciate or a continuous interlocking suture pattern is appropriate, making sure to apply moderate to marked tension on the sutures. In addition to intramuscular antibiotics and oxytocin, it is recommended to administer NSAIDs in order to prevent possible shock.

Maternal Recovery Rate and Causes of Death

The maternal recovery rate after Caesarean surgery in the sow is usually quite good unless the fetuses and uterus are grossly infected. In a series of 78 unselected cases, Renard et al. (1980) reported a maternal recovery rate of 72%. Deaths are typically due to the combined effects of toxaemia and surgical shock and occur during the immediate postoperative period. Animals that are likely to die can often be identified as such before surgery because of a characteristic blotchy cyanosis of the limbs, ears, and udder. The adverse effects of peritoneal contamination are more easily avoided in the sow than in larger animals because the uterus can be exteriorized in its entirety during the surgery. Other frequent complications reported by Renard et al. (1980) include constipation, locomotion problems exacerbated by the sow's tendency to remain recumbent, and the mastitis–metritis–agalactia syndrome. Severe preoperative vaginal prolapse may recur after surgery and requires the insertion of a temporary retaining perivaginal suture.

Llamas and Alpacas

Nearly all information in this chapter relates to both the domesticated species of llama and alpaca. Differences are only the relative shorter normal gestation length of alpacas, and the smaller body size, birth canal, and term fetus (cria) of the alpaca (Johnson 1997). The birthing process in these camelids is quick and, compared with other farm animals, dystocia is an uncommon event. Studies in South America found that less than 2% of births required assistance and that 25% of these occurred in primiparous females (Bravo 2002). Caesarean surgery is indicated if labour has been protracted, fetal heart rate cannot be assessed, or the condition of the fetus or birth canal prohibits vaginal delivery. Unlike cattle, fetal maturity is highly variable in llamas and alpacas. For this reason, it is inadvisable to carry out an elective Caesarean operation in these species. If maternal conditions require surgical intervention (e.g., fractured pelvis), it is recommended to allow the dam to enter Stage I labour first.

Indications

The most common causes, and therefore indications, for Caesarean surgery in llamas and alpacas are in order of importance:

1. Faulty fetal disposition, with 70% of these occurring with the fetus in anterior presentation and 30% in posterior presentation (Bravo 2002)
2. Uterine torsion
3. Poor cervical dilation

Malpositioning of the fetal head (deviation to the side, to the back, or down) can be associated with rotation of the long neck, which is then commonly impacted into the pelvic inlet. Fetotomy or Caesarean surgery is needed to complete delivery of the fetus if this faulty disposition is further complicated by fixation of the cervical vertebrae ('wryneck'). Occasionally, Caesarean surgery may be necessary because of disparity between the dam's birth canal and the fetus. Most dystocias due to fetal malformation (e.g., schistosoma reflexus, ankylosis) require Caesarean hysterotomy.

Anaesthesia and Restraint

Because the majority (> 95%) of fetuses reside in the left uterine horn (Johnson 1997), restraint in right lateral recumbency and using a left paralumbar fossa incision seems appropriate. The ventral midline approach has also been recommended but is best carried out with the dam under general anaesthesia (Fowler 1998). With the dam sedated and restrained in right lateral recumbency, crias are likely to be more vital and maternal-neonate bonding also occurs more quickly (Anderson 2009).

Intravenous administration of butorphanol (0.03–0.1 mg/kg) will render most adult females very manageable. If necessary, based on maternal activity, xylazine (0.1–0.3 mg/kg) may also be used; this sedative can be readily reversed using tolazoline or yohimbine. In cases in which the animal is intractable, deep sedation may be necessary using a combination of ketamine (4.4 mg/kg) and xylazine (0.44 mg/kg), both given intramuscularly.

Operative Technique

The dam is haltered and positioned in right lateral recumbency, with the head and limbs safely tied to prevent unnecessary movement. After clipping and surgical preparation, lignocaine 2% is used to establish a line block at the site of the incision. It is important not to exceed 4 mg/kg total dose of lignocaine (1 mL/5 kg body weight) so as not to induce lignocaine toxicity (Anderson 2009). A 15-cm cranioventral (oblique) skin incision is made from approximately 8 to 10 cm cranial and ventral to the tuber coxae towards the last costochondral junction. After careful incising the three flank muscles, the peritoneum is pinched, lifted, and cut with scissors. With the left flank approach, compartment 1, spleen and left kidney are located against the abdominal wall and may be accidentally cut during entry into the abdominal cavity. The uterus is exteriorized through the flank incision and the fetus removed. In llamas and alpacas the uterine wall is surprisingly thin and care

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should be taken when opening the uterus so as not to cut into the fetus. Usually, the placenta is not easily removed and is best left in place to avoid excessive endometrial haemorrhage (Anderson 2009). A healthy uterus may be closed in a single layer with 0 USP (3.5 metric) plain catgut or a comparable synthetic suture material such as polydioxanone (PDS II suture). In case of an infected or otherwise compromised uterus, a double layer closure should be done to obtain an adequate serosal seal. Before returning the uterus into the abdominal cavity, any blood clots and fibrin are carefully rinsed off. Compared with ventral midline incisions, paralumbar incisions in llamas and alpacas carry a greater risk of postoperative incisional herniation. Therefore each of the muscle layers should be exactly restored with simple continuous suture patterns, using 1 USP (4 metric) polyglycolic acid suture (Anderson 2009). Chromic catgut or polydioxanone are good alternatives. Finally, the skin incision is closed in an interrupted or continuous, interlocking suture pattern, using 1 USP (4 metric) nylon or polypropylene suture material.

Broad spectrum antibiotics and NSAIDs should be routinely administered before surgery and continued for at least 3 days after surgery – longer if required. The use of oxytocin has been associated with abdominal pain in llamas and alpacas. Therefore caution is advised, and oxytocin should only be administered if the cervix is open (5 IU subcutaneously, every 2–4 hours, repeated 2–4 times).

Postoperative Fertility

Published information on success and fertility rates after Caesarean hysterotomy in llamas and alpacas is very limited. The rebreeding success rate is considered to be good, as long as the surgery is done early in dystocia and a sterile technique is used. Under these circumstances, Anderson (2009) expected that 75% of females that had had Caesarean surgery would return to normal breeding soundness.

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Disorders of Parturition and the Puerperium in the Gilt and Sow

OLLI AARNO PELTONIEMI, STEFAN BJÖRKMAN AND CLAUDIO OLIVIERO

'Dystocia' or difficult birth is discussed across the species in Chapters 11 and 12, in which obstetrical terminology is defined, whereas the puerperium in all species is discussed in Chapter 7. The emphasis in both these chapters is on monotocous species, such as cattle and horses; the circumstances in polytocous species such as the pig are very different.

In the wild pig, which is considered to be the ancestor of present day farmed pig breeds, the duration of farrowing is short (1–1.5 hours), but the litter size is much small than that of the high prolific commercial sow lines used commercially today. In these, farrowing lasting longer than 5 hours is the norm (Björkman et al. 2017), whereas 10 years ago it was the upper limit for a normal successful farrowing (Oliviero et al. 2008).

Overview: Causes and Incidence Rates of Porcine Dystocia

It is generally accepted that the shape of the porcine fetus and its small size in relation to the size of the pelvic inlet of the mother make the incidence of dystocia in sows lower than in other farm animals. Reports are: 0.25% (Jones 1966), 1% (Jackson 1972), 3 % (Randall 1972), and 5% (Waldmann 1993). A survey by Jackson (1972) showed that dystocia was of maternal origin in about 63% and of fetal origin in about 37% of the cases. Maternal causes were mainly uterine inertia (37%), obstruction of the birth canal (13%), and downward deviation of the uterus (9.5%). The relative incidence of the different forms of uterine inertia were: primary (20%), secondary (49%), and idiopathic (31%) inertia. Fetal causes were mainly faulty disposition (33.5%) and oversize (4%). Faulty disposition was caused by breech presentation (14%; faulty posture with both hindlimbs extended forward with the fetus in posterior longitudinal presentation), simultaneous presentation (10%), and postural defects in anterior presentation such as downward deviation of the head (3.5%). In another survey (Wehrend 2003) dystocia was 63% of maternal and 37% of fetal origin. Maternal causes were mainly due to uterine inertia (32%), oedema of the birth canal (9.5%), vaginal prolapse (7%), vaginal injuries (5.5%), obstruction of the birth canal (4%), uterine torsion (2.5%), and bladder flexion (1.5%). Fetal causes were mainly due to fetomaternal disproportion (26%) and faulty disposition (11%). Interestingly, a survey in Germany between 1982 and 1988 (Waldmann 1993) identified that dystocia was almost exclusively due to maternal causes: 78.6% of dystocias were caused by obstruction of the birth canal, 11.4% by prolapsed vagina, 4.2% by primary uterine inertia, 3.9% by uterine torsion, 0.7% by downward deviation of the uterus, and 0.5% by a prolapsed uterus; only 0.4% were caused by piglet oversize. In this survey, secondary uterine inertia was not considered as a cause. Another survey (Schulz & Bostedt 1995) showed that bladder flexion caused 3.1% and vaginal prolapse 6.3% of dystocias. Dystocia may also occur as a result of the use of prostaglandin F2α and oxytocin to induce or control parturition (see Chapter 8).

Dystocia Due to Obstructive Causes

Vaginal Prolapse. Vaginal prolapse (see Chapter 10) was the cause of dystocia in 11.4% of the cases in the study by Waldmann (1993), in 7% by Wehrend (2003), and 6.3% by Schulz and Bostedt (1995). In the latter study, 78.8% of vaginal prolapses occurred within 1 week before parturition and mostly in sows in their third or more pregnancy. In 21.2% of the cases there was concurrent rectal prolapse. The aetiology is not clear, but it is likely that hormonal imbalances, increased abdominal pressure, and/or very relaxed birth canal caused by instability of pelvic ligaments and/or connective tissue of the perineal area play a role. The vagina can be partially or totally prolapsed, which can be temporary or permanent. If the vaginal prolapse does not resolve spontaneously, treatment is indicated. Otherwise, swelling and oedema of the vaginal mucous membranes can stimulate further abdominal straining and therefore increase the risk of rectal prolapse and vaginal injury with subsequent infection.

Bladder Flexion. Jackson (1972) reported that bladder flexion can cause obstruction of the birth canal resulting in dystocia; he identified it in 13% of the cases. In the survey by Schulz and Bostedt (1995), bladder flexion was associated with 3.1% of dystocias and in the study by Wehrend (2003) with 1.5%. Bladder flexion occurs mainly in multiparous sows in their third or higher pregnancy (Schulz & Bostedt 1995, Jackson 2004) and within 1 week before parturition (50% of all bladder flexions in the survey by Schulz and Bostedt (1995)) or during parturition (Jackson 2004). It can be accompanied by rectal prolapsed, as it was the case in 31.2% of dystocia caused by bladder flexion (Schulz & Bostedt 1995). The aetiology of both disorders is similar. Jackson (1995) reported that it occurred in sows with a very relaxed birth canal and surrounding tissue, together with excessive straining.

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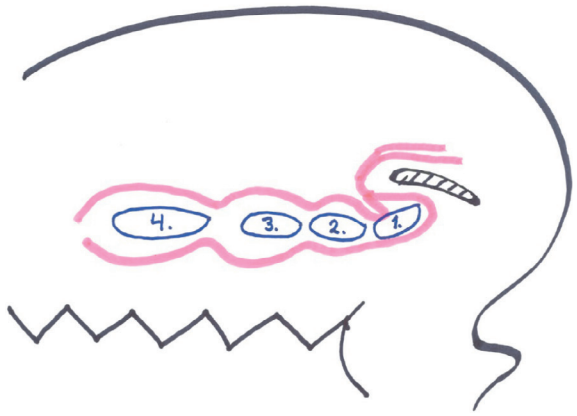

• **Fig. 17.1** Downward deviation of the uterus. The most caudal piglets *(piglets 1. and 2.)* create a bend in the uterine horn under the pelvic bone, creating a downward flexion of the horn and thus causing its obstruction.

The latter probably caused kinking of the urethra, resulting in urinary obstruction and bladder distention due the accumulation of urine. The distended urinary bladder was then further forced back into the pelvic cavity and thus ventral to the vaginal floor, causing it to be elevated and forming a 'mound', thereby obstructing the birth canal.

Downward Deviation of the Uterus. Downward deviation of the uterus occurred in 9.5% of dystocia cases (Jackson 1995) or only in 0.7% as in the study by Waldmann (1993). The incidence seems to be lower than that of both vaginal prolapse and bladder flexion, though it might be greater in modern highly prolific breeds. Affected animals strain vigorously despite an empty vagina, and at a point in front of the pelvic brim, the uterus deviates sharply in a ventral and caudal direction under the pelvic brim (Fig. 17.1). Affected sows are multiparous, with deep bodies and large litters (Jackson 2004). Causes may be primary uterine inertia and atony of the uterine muscle (Waldmann 1993).

Uterine Torsion. In a uterine torsion the uterus rotates on its long axis. No cases of torsion were reported in the study by Jackson (1972), but there were 3.9% and 2.5%, respectively, in the studies of Waldmann (1993) and Wehrend (2003). It appears to develop shortly before parturition (Waldmann 1993) and mainly in multiparous lean sows. The rotation can involve the uterine body, one uterine horn, or part of one uterine horn, thereby trapping a fetus or fetuses distal to the stricture. The torsion can be clockwise or anticlockwise, and the degree of torsion can be of 180 degrees to 360 degrees, although a complete torsion is very rare (Waldmann 1993). The uterine wall may rupture, and a fetus or fetuses may become pseudoectopic. Moreoften the torsion resolves spontaneously during parturition without any adverse effects.

Obstruction Due to Other Soft Tissue Abnormalities. Other obstructive causes are rare and variable. Furthermore, the precise classification is not always clear in published studies. Incomplete dilatation of the cervix during the first stage of labour seems to be uncommon. When it does occur, it may be the result of an antepartum infection of the placentae or the uterus. Incomplete dilatation at the end of the second stage of labour may become relevant in sows with prolonged parturition. Involution of the cervix can start 6 hours after the beginning of the second stage of labour. Although the cervix will be still open, the loss of elasticity, stretchability, and lubrication can lead to complications for the piglets entering and passing through the birth canal. Because in modern highly prolific sows the duration of parturition is on average 6 hours (Björkman et al. 2017), this cause of dystocia may now be more relevant.

Another soft tissue abnormality that may be increasingly relevant is severe swelling and oedema of the birth canal which can occur in prolonged parturitions, after an obstetrical examination, and/or piglet removal, particularly if the procedure is not done gently (Björkman 2017). The birth canal is very delicate, fragile, and susceptible to trauma. In cases in which there are a large number of piglets entering the birth canal simultaneously followed by manual extraction of piglets, the mucous membranes may react with increased swelling. Furthermore, the mucous membranes can be more easily damaged. Thus in sows with large litters and prolonged parturition, there is an increased need for manual interventions, and dystocia caused by swelling and oedema of the birth canal may be more relevant. In addition, there is evidence that sows with a large litter also have higher oestrogen concentrations in the blood (Edgerton et al. 1971, Kensinger et al. 1986), especially before parturition, resulting in severe oedema of the vulva (Waldmann 2009).

Obstruction Due to Bony Tissue Abnormalities. Obstruction caused by bony tissue abnormalities, e.g., pelvic constriction, are very rare and only cause dystocia when they occur in gilts which have been bred at a too young age (< 200 days) and a too low body weight (< 90 kg) (juvenile pelvis); they rarely occur in sows after fractures, in which there has been poor alignment of the pelvic bones. Gilts should be between 220 to 270 days old, and a body weight of 135 to 170 kg, a backfat depth of 12 to 18 mm, and a body condition of about 3, when they are bred for the first time (Rozeboom 2015). If these recommendations are followed, the incidence of dystocia due to a juvenile pelvis should be very low.

Obstruction Due to the Piglets. Jackson (1972) reported that 37.5% of dystocias were due to a fetal cause. These were due mainly to faulty fetal disposition (33.5%) and oversize (4%). In the study by Waldmann (1993) no fetal causes of dystocia was reported. 57% of the piglets were born in anterior longitudinal presentation, whereas 43% were born in posterior longitudinal presentation. Posterior presentation is not itself a cause of dystocia, unless the piglet is oversized. Currently, fetal causes of dystocia are minimal. It may occur in gilts bred at too young an age or with low body weight (see previous mention), or in which the litter is small. Infection, with porcine parvo virus, can cause small litters. In sows with large litters the smaller piglets can be expelled even when their fetal disposition is incorrect and as a result, simultaneous presentation of two piglets may become more frequent. Congenital abnormalities are very rare; they may include: arthrogryposis, hyperostosis, hydrocephalus, conjoined piglets, schistosoma reflexa, syndactyly, polydactyly, and micromelia (see Chapter 9). Arthrogryposis is characterised by ankylosis of various joints, in various degrees of flexion or extension, as well as the vertebral column resulting in lordosis, kyphosis, or scoliosis. Hyperostosis is stiffness of the forelegs, with enlarged elbow joints and cartilaginous thickening of proximal bones of the forelimbs.

Inadequate Expulsive Force

Primary Uterine Inertia. Primary uterine inertia is defined as the absence of or deficient myometrial contractions (see Chapter 13). Primary uterine inertia, which is the absence of or reduced contractility of the myometrium, was present in 7.5% of dystocias (Jackson 1972) and in 4.2% in Waldmann's study (1993). In toxaemic sows there may be signs of systemic illness before farrowing

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with a foul-smelling vaginal discharge, which is sometimes associated with infections such as porcine parvovirus. Nontoxaemic sows are usually healthy, with a dilated cervix and fetuses palpable within the birth canal but with the absence of contractions. It may be caused by hormonal abnormalities, such as an increased progesterone to oestrogen ratio, and/or deficiencies of oxytocin and prostaglandin secretion and/or their receptors, and nutritional factors, for example diets low in fibre and high in energy. Other less common causes, such as calcium deficiency, have been reported (Framstad et al. 1989). The inability of sows to be able to express normal nest-building behaviour may be an important primary cause of uterine inertia. It has been shown that lack of space and absence of available nest-building materials can be an important stressor in sows, thereby decreasing oxytocin secretion, and the resultant reduced uterine contractility will eventually increase the duration of farrowing (Oliviero et al. 2008).

Secondary Uterine Inertia. Secondary uterine inertia is more common than primary inertia, usually occurring as a result of another cause of dystocia, usually obstructive but also associated with a prolonged parturition particularly associated with a large litter.

Management

Inappropriate Use of Exogenous Oxytocin. Oxytocin is widely used to augment normal uterine contractions. Its uterotonic has been demonstrated by Mota-Rojas et al. (2005a), who showed that exogenous oxytocin increases the number, intensity, and duration of myometrial contractions. However, its use has been shown to have negative effects on the outcome of farrowing, for example increased incidence of intrapartum asphyxia and stillbirths (Mota-Rojas et al. 2002, Alonso-Spilsbury et al. 2004, Mota-Rojas et al. 2005b) and the duration of piglet expulsion. Several studies (Welp et al. 1984, Chantaraprateep et al. 1986, Dial et al. 1987, Alonso-Spilsbury et al. 2004) have shown that oxytocin can increase the frequency of dystocia and therefore the need for subsequent obstetrical assistance. However, most of the adverse effects have been associated with its use when not truly indicated, e.g., at the beginning of fetal expulsion when oxytocin concentrations and oxytocin binding sites in the endometrium and myometrium are high (Lundin-Schiller et al. 1996, Phaneuf et al. 2000). The time of administration is appropriate when the expulsion of the last piglet was at least 30 minutes ago, and if the obstetrical examination showed that piglets are still present within the uterus but none are within the birth canal. Furthermore, the birth canal should be fully dilated and unobstructed.

Body Condition. There is a negative correlation between the amounts of backfat and farrowing duration (Oliviero et al. 2010). The fatter the sow, the longer the duration of farrowing. The reason may be that increased backfat reflects the level of fatty infiltration of the tissues of the birth canal, resulting in a reduction in the diameter of the birth canal, as well as predisposing to secondary uterine inertia. Another reason may be that a higher level of fat is known to affect lipid-soluble steroids and may affect the progesterone to oestrogen ratio, a consequence of which is that it affects oxytocin receptor activation. Abnormalities due to oxytocin receptor activation may weaken the expulsive phase of parturition. This hypothesis is supported by the observation of Oliviero et al. (2008), who observed that fat sows had a delayed decline in peripheral progesterone concentrations beyond day 1 after parturition. Furthermore, Björkman et al. (2017) observed a negative correlation between backfat and the depth of the uterine wall antepartum, which could be the result of a delayed decline in progesterone or a higher progesterone to oestrogen ratio, which could impair the degree of myometrial hypertrophy during pregnancy and hence before parturition.

Constipation. There is a negative correlation between constipation and farrowing duration (Oliviero et al. 2010). The more constipated the sow, the longer the duration of parturition. The reason may be that constipation results in an accumulation of faeces in the rectum that causes a physical obstruction to the passage of the piglets, resulting in secondary uterine inertia. Another reason may be that constipation may result in impaired uterine contractions due to the effects of lipopolysaccharides. Lipopolysaccharides are endotoxins produced by Gram-negative bacteria that increase around the time of parturition and are associated with constipation. Endotoxins can be absorbed from the gut and influence the normal endocrine changes associated with farrowing, resulting in primary uterine inertia. A third explanation may be that the discomfort and pain associated with constipation may also influence the hormonal changes associated with parturition. Studies have found that opioids inhibit oxytocin during parturition (Bicknell & Leng 1982, Douglas et al. 1995. Brown et al. 1999). Therefore pain due to prolonged constipation could promote the release of opioids and, consequently, impair oxytocin secretion, thus reducing myometrial contractions.

Dystocia: Examination

History of the Sow. Before examining an individual sow with suspected dystocia, it is important to obtain details of her previous farrowing history, if appropriate, by examining her records for breeding and obstetrical history; these may be manually or electronically recorded. Details include general health, number of litters and their sizes, and duration of previous gestations.

Duration of Farrowing. The timescale before intervening in the process of parturition is critical. Knowing the normal physiology and behaviour of the particular sow and her immediate cohorts is important. Previous studies (Oliviero et al. 2008) have reported the average values for the duration of farrowing were 200 to 300 minutes, which were considerably longer than those reported earlier. However recent data suggests that with ever increasing litter numbers, there is a concomitant increase in the duration of farrowing. Björkman et al. (2017) have recently reported that in highly productive sow lines, the average duration of farrowing was 400 minutes, which was influenced by parity and number of stillborn piglets. Thus classifying a sow as suffering from dystocia according to the 5-hour principle as occurred 10 years ago is not applicable now. By providing the sow with an open farrowing pen and adequate amounts of suitable nest-building material, and dispensing with farrowing crates, reduces the duration of farrowing by approximately 90 minutes (Oliviero et al. 2008).

Inter Piglet Interval. The decision on when it is appropriate to intervene will depend on a number of factors, e.g., if the interpiglet interval is prolonged at the beginning and end of the second stage of parturition, if the piglet fetus is large, especially some males, and if it is dead.

General Examination. Frequent observations of the sow before farrowing should enable the signs of impending farrowing to be detected. General examination may include measuring body temperature; it normally increases above 39°C 24 to 48 hours before the birth of the first piglet and heart rate peaks before and during parturition. Nesting behaviour is greatest 6 to 12 hours before the first piglet is born. Palpation of the mammary gland for the composition and presence of colostrum, which normally runs

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freely during parturition, appearing first in the cranial pair of the mammary glands some hours before beginning of parturition. After the onset of the second stage of labour and as it progresses, the number of piglets born, as well as their vitality and uniformity, should be assessed (Oliviero 2010).

A lack of uniformity in the weight of new born piglets may be an indicator for a large litter; low piglet vitality may indicate a prolonged inter piglet interval. Large litters may result in uterine inertia, and prolonged interpiglet intervals may be indicative of endocrine imbalances or obstructive dystocia. Continuous straining by the sow without piglet expulsion, bleeding, or an abnormal vaginal discharge, including meconium staining of the piglet as well as loss of movement immediately after birth (see piglet vitality scoring later in this chapter) are indicative of disturbances of farrowing.

Intervention Criteria. As discussed previously, if the inter piglet interval is longer than 2 hours or the overall duration of farrowing is longer than 5 hours, it is indicative of dystocia. Although the criteria for intervention may have changed in recent years, intervention should always be based on the history and health of the sow and the piglets that have been born.

Obstetrical Examination

Rectal Palpation. This should be considered before the vagina is examined for number of reasons. Provided it is performed carefully, it is easily performed, using a gloved hand and plentiful lubricant, and safe. By performing the procedure, one of the potential causes of obstruction of the birth canal, namely a hard faecal mass due to constipation can be removed. It also reduces the likelihood of vaginal faecal contamination during subsequent vaginal examination. Rectal palpation has limitations because it is only possible to reach the body of the uterus and caudal parts of the uterine horns.

Vaginal Examination. This should be done carefully and cleanly using a gloved hand/arm and with plentiful amounts of lubricant. Once the hand is in the birth canal, the mucosa of vagina, cervix, and uterine body should be carefully palpated for any signs of possible damage, including swelling of the mucosa, which readily occurs after an obstructive dystocia. Cranial to the uterine body, the caudal parts of the both uterine horns should be palpated to determine the presence of downward, ventral torsion of uterine horns, which is typical in a sow with a large litter.

Ultrasonographic Examination. Real time transabdominal ultrasonography is very useful as part of an obstetrical examination to identify the number of fetuses present within the uterus, as well as detecting their heartbeats, thus indicating the number that are alive.

Dystocia: Treatment

Uterine Inertia. As described previously, this is the commonest cause of dystocia, occurring in over a third of cases. Clinical examination will show that no fetuses can be palpated in the birth canal, and uterine contractions cannot be detected. Initially, if possible, the sow should be encouraged to stand and move around even outside the farrowing pen. The sow should be treated with oxytocin at a dose of 5 to 10 IU i.m for a maximum of 2 to 3 times; the time interval between the successive injections should not be less than 30 minutes. As a last option, manual palpation of the cervix may be tried to stimulate the release of endogenous oxytocin.

Use of Oxytocin in Prolonged Farrowing. In prolonged farrowings, the overuse of oxytocin may expose those fetuses still within the uterus to hypoxia because oxytocin reduces blood flow through placenta and also increases the risk of a ruptured umbilical cord and thus the severance of blood supply to the fetus. It is well documented that piglets appear vulnerable to asphyxia during parturition, and the increasing litter size appears as a common denominator for this.

Downward Deviation/Torsion of the Uterine Horn. Large litters seem to predispose the sow to downward deviation/torsion of the uterine horn (Figure 17.1). In the sow, in contrast to other larger domestic species, the twisting of the caudal part of the uterine horn is due to the caudal fetuses becoming positioned ventral to the pelvic bone.

Prevention of Dystocia

As described previously, dystocia in sows is relatively rare compared with other multiparous species. Dystocia adversely affects piglets' survival, thus there is an increase number of stillborn piglets as well as those that are considered to be 'hypovital' (Alonso-Spilsbury et al. 2007). It also affects the survival of the sow (Sanz et al. 2007), as well as her subsequent fertility (Oliviero et al. 2013). For all these reasons, it is important to attempt to prevent dystocia throughout the course of pregnancy until the start of farrowing. For example, maintaining good body condition and preventing constipation are important. These factors, unlike mechanical and obstructive causes of dystocia, can be influenced to some extent by proper management and feeding strategies.

Endogenous Hormonal Balance at Farrowing

Modern housing and production systems have promoted the confinement of sows in crates during farrowing, whereby the sow's movement is severely restricted, and she is allowed limited bedding. As a consequence, nest-building at the best is limited or does not occur at all. The sow's innate behaviour of nest-building is induced by the decline in progesterone and the increase in prolactin prepartum (Algers & Uvnäs-Moberg 2007). As well as these endocrine changes, 6 to 12 hours before farrowing oxytocin increases before peaking at parturition to stimulate effective contractions of the uterus and, as a consequence, rapid birth of the piglets (Taverne et al. 1979, Lawrence et al. 1997). There are studies showing an increase in farrowing duration and stillbirth rate when innate behaviours, such as restricting nest-building by the absence or limited availability of the necessary materials, as well as limiting the mobility of sows by confinement and restraint by the use of the farrowing crate (Oliviero et al. 2008 and 2010, Gu et al. 2011). Allowing the sow to move freely before and during farrowing reduces the duration of farrowing by an average of 100 minutes, thereby reducing the risk of stillborn piglets. A combination of a free pen and the provision of abundant nest-building material increased the circulating level of oxytocin and prolactin before farrowing (Oliviero et al. 2008, Yun et al. 2015). Therefore it is recommended that sows are allowed to move freely to perform natural nest-building behaviour and to provide nest-building material such as straw, hay, sawdust, and paper sheets. This will help to ease the parturition process, reducing risks of prolonged farrowing that may have negative consequences for the sow and her offspring.

Feeding in Late Pregnancy

During late pregnancy, to ensure that sows receive enough energy to satisfy the demands of forthcoming milk production, sows

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are usually fed with high energy concentrated diets (Einarsson & Rojkittikhun 1993). Such concentrated diets, which have a much more limited amount of fibre than standard pregnancy diets, can promote obesity and constipation. These two conditions are associated with prolonged duration of farrowing and an increased stillbirth rate (Oliviero et al. 2010). Late pregnancy diets should contain up to 7% to 10% of fibre, in order to reduce constipation and excessive fat deposits (Oliviero et al. 2009). The use of high fibre diets appears to be a beneficial strategy to improve the health of the sow around farrowing (Peltoniemi et al. 2016). If the diet cannot be easily modified, a good fibre source can be provided by offering different types of roughage (straw, hay) or adding any other feedstuffs with high levels of fibre, such as sugar beet pulp (Quesnel et al. 2009). Offering roughage may not only be a way to increase fibre intake and alleviate constipation and obesity, but it can also serve as an appropriate material for nest-building. Preventing sows becoming excessively fat in the last part of pregnancy can be achieved by encouraging sows to be more mobile by allowing them more space, with strategic feed distribution and with provision of interactive substrates like straw or hay. A reduction in feed intake by the sow (up to 50%) may be implemented during the last week before farrowing, which also helps to prevent constipation, as well as other physiological and behaviour aberrations around the time of farrowing.

Disturbed Parturition and the Neonatal Piglet

Introduction

During the last two decades there has been emphasis on the development of highly prolific pig breeds in many countries, resulting in substantial increases in litter size (Bjerre et al. 2010, Paredes et al. 2012). In order to improve the productivity of sows, a better method would be to select breeding stock based upon the number of piglets weaned. However, due to the common practice of crossfostering small piglets at an early age of life, the total number piglets born has been the preferred method of selection (Su et al. 2007). Litters with greater than a total of 16 born piglets have been associated with higher stillbirth rates (1.5 piglets) compared with litters of 11 to 12 total born piglets (0.6 piglet), and piglets in larger litters also have lighter birthweights (Boulot et al. 2008). Comparing similar litter sizes, Canario et al. (2006) found the probability of stillborn piglets in large litters to be almost twice as high as in a reference litter. Large litters are also associated with longer farrowing times, a higher risk of fetal hypoxia during birth (Herpin et al. 2001), and higher within litter weight variation (Milligan et al. 2002). Piglets' mortality after birth and before weaning have ranged from 11% to 24% in several countries, and the mortality is mostly observed in the first 5 days of life (Pedersen et al. 2011, KilBride et al. 2012). The main causes of death included crushing, low viability, starvation, and diseases. These levels of preweaning mortality represent a challenge to modern pig production and also a concern for animal welfare.

Weak and Stillborn Piglets: Causes and Consequences

Different studies have reported that stillbirths rates vary between 3% to 10% of all pigs born (Borges et al. 2005, Cutler et al. 2006, Oliviero et al. 2010, Vanderhaeghe et al. 2010). According to the time of death, stillbirths can be classified into: type I, in which fetuses die before the end of gestation (antepartum or prepartum deaths), due to infectious causes; type II, in which piglets die during parturition (intrapartum deaths) due to noninfectious causes, such as intrauterine asphyxia and dystocia (Sprecher et al. 1974).

TABLE 17.1 Number of stillborn piglets according to housing type (n = litters)[a]

Housing	Number of Stillborn	Average Duration of Farrowing (min)	n
Pen	0.6 ± 0.8	208 ± 58	89
Crate	1.1 ± 1.1	297 ± 130	133

[a]Summary of three studies (Oliviero et al. 2008, 2010; Gu et al. 2011).

One cause of type II stillbirths is large litter size (> 16 total born piglets) as previously discussed. Another cause is housing. Both housing and stillbirth rate relate to farrowing duration (Table 17.1). Farrowing duration > 300 minutes was associated with a higher stillbirth rate, compared with the duration of farrowings of <200 minutes (Oliviero et al. 2008 and 2010, Gu et al. 2011). According to the review by Vanderhaeghe et al. (2013), large litter size, high parity, excessive thin or fat sow's body condition, and limited or no farrowing supervision/birth assistance seem to be the most relevant factors in increasing the stillbirth rate.

Thermoregulation is challenging for neonate piglets, and this competence is highly correlated with their vitality after birth. They are born wet, covered by fetal fluids that allows for heat loss by evaporation, so it is important that they quickly dry. They have very little pelage to insulate against heat loss, and they lack brown fat that could be metabolised to generate heat and balance their body temperature (Berthon et al. 1994). Thus newborn piglets are very vulnerable to the effects of environmental temperature and can only use metabolic responses to reduce heat loss if their body temperature is >34°C (Lossec et al. 1998). The main metabolic responses are mobilisation of free fatty acids, shivering, and a modest gluconeogenesis using their own liver's glycogen reserve. The latter is available and metabolised within a maximum of 15 to 20 hours in starving piglets (Svendsen & Bengtsson 1986, Lossec et al. 1998). Pedersen et al. (2011) found that piglets with a rectal temperature of 36.1°C, 2 hours after birth, had greater odds of being crushed by the sow, whereas in those with a rectal temperature of 38.6°C, the odds were reduced.

Herpin et al. (1996) demonstrated that piglets that suffered from hypoxia due to rupture of the umbilical cord or detachment of the placenta during parturition were less viable and needed more time to reach the sow's udder (120 ± 35 vs 25± 3 min). They also showed a reduced ability to maintain the body temperature (36.3 ± 0.9 vs 38.4 ± 0.1°C) during the first 24 hours of life. In contrast to older animals, the early neonatal piglet does not increase its energy intake in response to cold temperature. In fact, colostrum intake decreases by 36.8% during cold exposure (18° – 20°C) compared with piglets at an ambient temperature between 30° to 32°C, thus exacerbating the likelihood of starvation (Lay et al. 2002). Kammersgaard et al. (2011) found that a birth weight >1.1 kg was the most important single factor in successful recovery from postnatal hypothermia. In addition, Milligan et al. (2002) found that birth weight >1.3 kg was positively correlated with higher piglet preweaning survival. It has been shown that the birth process and individual characteristics influence the piglet's

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thermoregulatory mechanism. However, in the environment of a modern farrowing unit, with different zones at different temperatures, it is heat preservation rather than mere heat production that determines thermoregulatory success and recovery from postnatal hypothermia (Kammersgaard et al. 2011).

Assessment of Neonatal Vitality

Piglet vigour postpartum, in addition to birth weight, is crucial if the piglet is to acquire colostrum and preserve homeothermy (Baxter et al. 2008). Piglet vigour appears to be independent of birth weight, suggesting that small, but proportional, piglets that display high vigour can survive the vulnerable perinatal period (Baxter et al. 2008). The same authors propose the use of a categorical scale to visually assess and score the piglets' vitality immediately after birth (Table 17.2), which can be predictive of the survival rate of the neonate piglets after birth. In large litters, piglets can suffer from intrauterine growth restriction (IUGR) during uterine development. These piglets have a 'dolphin-like' head shape compared with 'normal' piglets (Fig. 17.2; Hales et al. 2013). Piglets with a 'dolphin-like' head shape ingest insufficient amounts of colostrums and hence have lower glucose levels at 24 hours. In addition, they have less glycogen reserves in the liver (Amdi et al. 2013). These factors combined suggest that if piglets are born with a dolphin-like head shape, they have a lower chance of survival. Identifying IUGR piglets on their head shape and evaluating the vitality score at birth can provide a rapid and simple method of deciding which piglets need extra support to maximise neonatal survival (Amdi et al. 2013, Baxter et al. 2008).

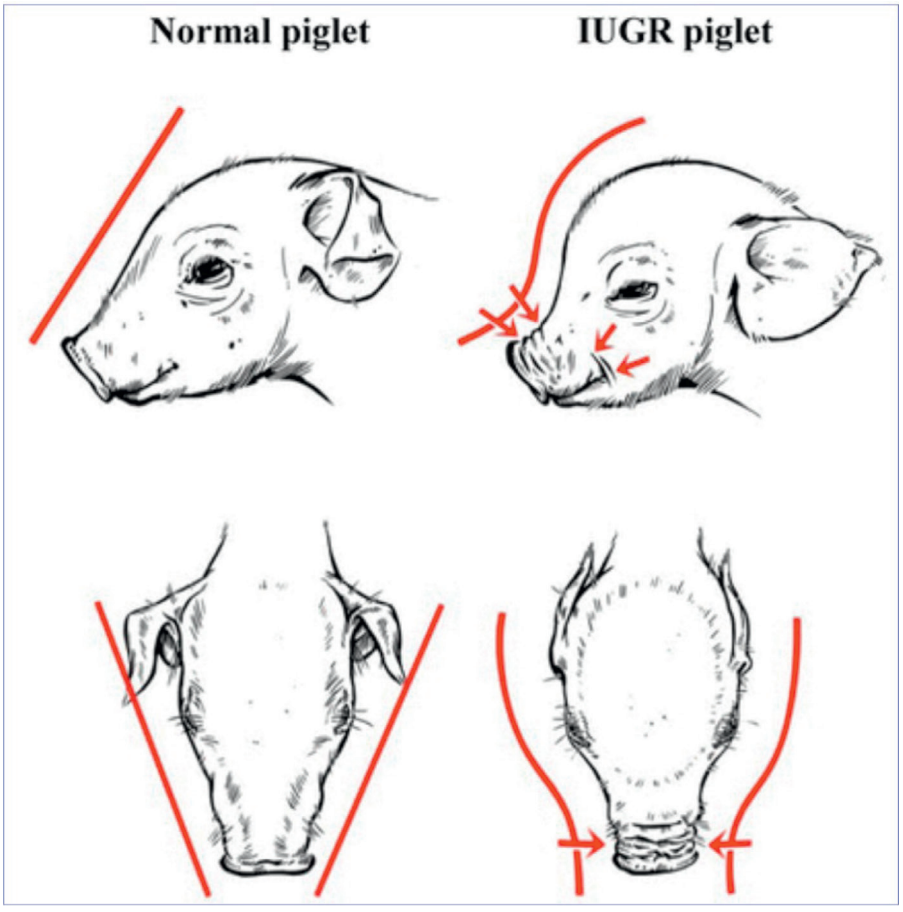
Management and Supportive Therapy of Neonates

Piglets should be quickly dried soon after birth and placed in a suitable environment to minimise their body heat loss. This can

TABLE 17.2 The vitality score of piglets immediately after birth

Vitality Score	Description
0	No movement, no breathing after 15 sec
1	No movement after 15 sec, piglet is breathing or attempting to breathe (coughing, spluttering, clearing its lungs)
2	Piglet shows some movement within 15 sec, breathing or attempting to breathe
3	Good movement, good breathing, piglet attempts to stand within 15 sec

Adapted from Baxter et al. 2008.



• **Fig. 17.2** Normal compared with IUGR piglet steep, dolphin-like forehead, bulging eyes, and wrinkles perpendicular to the mouth. (From Hales, J. et al, 2013. Individual physical characteristics of neonatal piglets affect preweaning survival of piglets born in a noncrated system. *J Anim Sci.* 91: 4991–5003.)

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be done using insulated nests, floor heating pads, heating lamps, or combinations of these. A second important step is to ensure that the whole litter has sufficient individual colostrum intake (200 – 250 g) within 12 to 16 hours from the beginning of parturition (Devillers et al. 2011). When possible, small and weak piglets should be assisted to suckle, by helping them to attach to the teat, thus ensuring that colostrum is ingested. It is important to remember that small piglets have difficulty in sucking from big teats, therefore the smallest functioning teats should be selected when assisting suckling. This procedure should be repeated 3 to 4 times within the first few hours if these piglets are not seen actively suckling. Additionally, weakly piglets can be hand fed with colostrum collected from their own mother, or other sows, within 6 to 12 hours from the beginning of farrowing. At this stage, milking by hand is relatively easy, due to the almost continuous (every 5–40 min) milk ejections (Algers & Uvnäs-Moberg 2007). Piglets can be hand fed using a feeding bottle with a suitable nipple or using a syringe connected to a soft silicone plastic tube (3–4 mm in diameter) that is inserted in the oesophagus and then stomach, and administering 20 to 25 mL of colostrum. The correct length of the tube can be assessed and subsequently marked, by measuring it against the piglet from the tip of its nose to the last rib. Great care is necessary to avoid intratracheal intubation insertion and injury to the larynx. Assisted suckling and hand feeding is appropriate in small or normal size litters when only one or two piglets require help. In large litters or when more piglets require assistance, a split suckling strategy could be more effective. In order to minimise the sibling competition for colostrum intake, the litter is split into two groups. The heavier and stronger piglets are kept in the creep area or in a separate box, allowing the smaller piglets to suckle for 60 to 90 minutes, and then the groups are switched. When separating the piglets, both groups should always have free access to a warm creep area. This can be easily achieved by using a box with an additional heat lamp for the separated group, which leaves the creep area accessible for the remaining group to suckle. If some small piglets are still unable to successfully suckle, assisted suckling should be combined with split suckling.

Colostrum Uptake

Because of the epitheliochorial nature of the porcine placenta (see Chapter 4), neonate piglets lack maternal IgG at birth. They must acquire maternal immunoglobulins from ingested colostrum for passive immune protection before they produce their own immunoglobulins at around 3 to 4 weeks of age (Rooke & Bland 2002). However, colostrum is produced only during the first 16 to 24 hours after the start of parturition, and even after the first 6 hours, the IgG content in colostrum is halved (Le Dividich et al. 2005). Acquiring adequate colostrum at the right time and in the required amount can be a challenge because of sibling competition, protracted farrowing, and reduced piglet viability. All these factors have a major effect in large litters (Devillers et al. 2007). The same authors found that individual colostrum intake ranged from nil to >700 g during the first 19 hours of life (450 g/kg birth weight), indicating that the capacity for colostrum intake can be high when its supply is unrestricted. In large litters, an ad libitum supply of colostrum is rarely available to all piglets, and probably 200 to 250 g intake per piglet provides sufficient protection. Devillers et al. (2011) found that the preweaning mortality rate was 7.1% when piglets ingested >200g of colostrums, and increased to 43.4% when intake was <200 g. Thus it is not the piglets' intake capacity that is the limiting factor in satisfying the passive immunity requirements, but the production of adequate colostrum by the sow. Colostrum yield is highly variable; it averages 3.3 to 3.7 kg, ranging from <1.5 kg to greater than 6.0 kg (Devillers et al. 2007, Quesnel 2011). However, Andersen et al. (2011) found that, without human assistance to piglets (such as drying, placing under a heat lamp, cross fostering, movement to a nurse sow, split- or suckling assistance), it can be difficult for many sows to adequately nurse more than 10 to 11 piglets. Furthermore, Quesnel et al. (2012) suggested that the genetic selection for higher prolificacy is one of the major causes for the increase in low birth weight of piglets and poor vitality. Insufficient colostrum production has been found to be associated with impaired hormonal status of the sow at farrowing. Abnormal high levels of circulating progesterone (< 4 ng/mL) in blood of sows at farrowing have been related to poor colostrum yield (Foisnet et al. 2010, Hasan et al. 2016a).

Evaluation of Colostrum Quality

Hasan et al. (2016b) have recently proposed the use of Brix refractometer to evaluate the IgG content of sows' colostrum content. Based on the study by Quesnel et al. (2015) and in the review by Hurley (2015), the IgG content in sows peaks within 2 hours after the start of farrowing, with a mean concentration of 64 mg/mL (range: 50–80 mg/mL), whereas it decreases to around 10 mg/mL at the end of colostrogenesis (24 hours after the start of farrowing). By using a Brix measurement on the farm, it might help to identify sows with an impaired IgG concentration and allow improved management of lactating sows and neonate piglets. This procedure should be done during early colostrogenesis (0–3 hours after start of farrowing), when colostrum is easiest to collect and when peak levels of IgG occur (Hasan et al. 2016). On the contrary, low IgG levels (10 mg/mL) are not expected to be found during early colostrogenesis. Taking the lower range of the peak IgG level as 50 mg/mL, it should be a fairly reliable point to assess that adequate levels of IgG are present at this stage. Table 17.3 shows a suggestion for the evaluation of the Brix refractometer results. Brix values <20% reflect very low levels of IgG, whereas values >25% reflect good or very good concentrations of IgG. Values between 20% and 24%, defined by the authors as borderline, should not be considered as being inadequate for the IgG content, especially if the Brix values are in the highest range (23% – 24%). Conversely, levels in the lowest range (20% – 21%) might be considered more critical. When borderline results are obtained, the authors suggest taking another sample within 1 to 2 hours, to evaluate if the development of the estimated IgG content is stable.

TABLE 17.3 Colostrum IgG content based on two methods of evaluation, and categories of estimation

Brix %	ELISA IgG 0–3h, mg/mL, average ± SEM	IgG estimation categories
< 20	14.5 ± 1.8	Poor
20–24	43.8 ± 2.3	Borderline
25–29	50.7 ± 2.1	Adequate
≥ 30	78.6 ± 8.4	Very good

From Hasan et al. 2016b.

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Abnormal Parturition and Reproductive Health

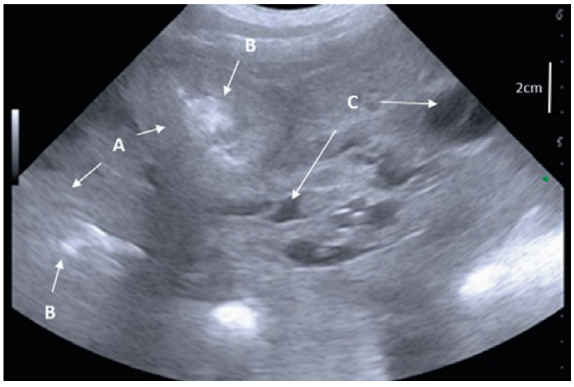
Effect of an Abnormal Parturition on Health of the Uterus and Mammary Gland Postpartum

There is enough evidence which suggests that a prolonged or abnormal farrowing predisposes the sow to poor reproductive health during the puerperium. When parturition is prolonged, placental retention is more likely to occur with consequential metritis (Björkman et al. 2017). It is also known that, if there is dystocia or farrowing is prolonged, there is an increased frequency of constipation and decreased circulating oxytocin concentrations (Oliviero et al. 2008), both of which predispose to disorders such as postpartum dysgalactia syndrome (PDS).

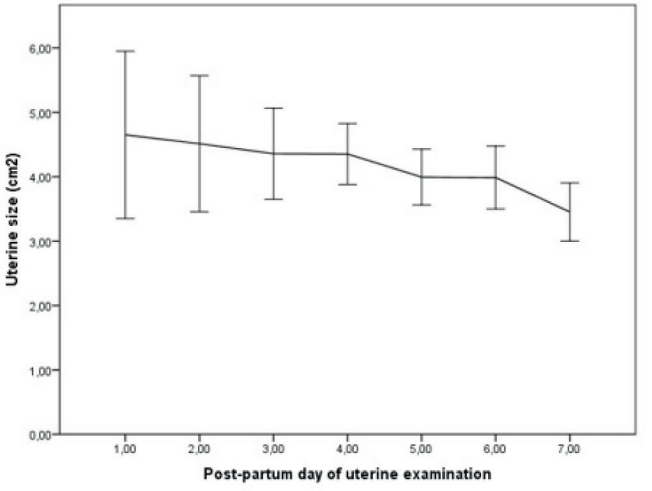
Retained Fetal Membranes. The time interval between the expulsion of the first and last placenta (placental expulsion duration) has been reported in sows as 4 hours and 4.5 hours (Björkman et al. 2017), and 2.5 hours in gilts (van Rens & van der Lende 2004). Van Rens and van der Lende (2004) showed that an increase in the duration of farrowing was significantly associated with an increase in placental expulsion duration. Björkman et al. (2017) showed that the duration of placental expulsion was mainly affected by the use of exogenous oxytocin during parturition. If the latter was used, then the litter size had the main effect on the duration of placental expulsion, probably due directly to the actual number of placentae that need to be expelled, which increases with increasing litter size. If exogenous oxytocin was not used, the farrowing duration was associated with the placental expulsion duration, confirming the observations made by Van Rens and Van der Lende (2004). Nevertheless, this association was observed only in sows with a farrowing duration of < 700 to 800 minutes. If the farrowing duration was longer, placental expulsion was severely compromised, which occurred in about 6% of the sows. Fifty percent of these sows had a partial retention of their placentae due to either a delayed onset of placenta expulsion and/or an early abortion. Another 50% of them expelled no placental parts and therefore had total retention (Fig. 17.3.); in these sows the average duration of farrowing was 1000 minutes. The most likely cause of impaired placenta expulsion in sows with a prolonged farrowing is secondary uterine inertia. This is supported by the findings of Oliviero et al. (2008) and Castrén and colleagues. (1993), who showed that increased farrowing duration is associated with decreased oxytocin levels. In addition, it is also supported by the observation that moderate use of oxytocin towards the end of the second phase of the parturition had a significant effect on reducing the duration of placenta expulsion (Björkman et al. 2017). If retained placentae can be expelled later during the puerperium, or are absorbed within the uterus, or expelled at the subsequent oestrus is not known.

Delayed Uterine Involution. Initially, uterine involution is very rapid during the first week postpartum (Fig. 17.4) when the uterine weight is reduced by 65% (Palmer et al. 1965). Thereafter, uterine involution continuous progressively and uniformly and is completed during the third week postpartum (Palmer et al. 1965, Kudlac & Groch, 1979, Schnurrbusch 1998, Busch 2007). During the initial period, uterine weight loss is mainly due to atrophy and resorption of intraluminal fluid, whereas after the initial period, uterine weight loss is mainly due to changes in the myometrium, notably a reduction in cell numbers, cell size, and amounts of connective tissue; this has been described as 'reorganisation' (Busch 2007). Björkman (2017) showed that transabdominal B mode ultrasound can be used to assess the course of the initial stages of uterine involution, by assessing uterine size, echotexture, and content (Fig. 17.5). Schnurrbusch (1998) defined a uterine weight of 500 to 700 g as being indicative of the completion of uterine involution, though Kauffold et al. (2005) and Busch (2007) reported a uterine weight of 1.5 kg. However, although uterine weight is an indicator of the successful completion of uterine involution, it is not necessarily a good indicator for subsequent fertility (Kauffold et al. 2005).

During the third week postpartum, many authors reported an increase in uterine weight for the length of the uterine horns (Busch 2007). Busch (2007) interpreted the increase in length and weight of the uterus during week three postpartum as tissue proliferation due to increased oestrogen production by developing follicles and considered that it was a sign of completion of uterine involution. Delayed uterine involution may cause subsequent fertility problems if sows are bred after a short lactation of, for example, 3 weeks. Björkman (2017) reported that prolonged parturition, multiple stillborn piglets, obstetrical intervention, and retained placentae can delay uterine involution. On the other hand, exogenous oxytocin



• **Fig. 17.3** Ultrasound image of retained fetal membranes in a sow. A, Uterine Horn; B, Uterine lumen and endometrium; C, Enlarged blood vessels. (From Björkman et al., 2017.)



• **Fig. 17.4** Decrease in uterine size (involution) as measured by ultrasound during the first week postpartum. (Adapted from Björkman, S., 2017. Parturition and subsequent uterine health and fertility in sows. (PhD thesis, University of Helsinki.)

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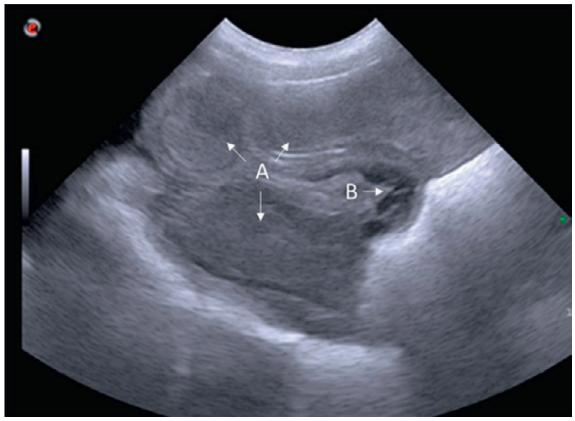

• **Fig. 17.5** Real time ultrasound image of a sow 3 days postpartum. *(A)* Enlarged uterine horn. *(B)* Enlarged blood vessels. (From Björkman et al, 2017.)

hastens uterine involution (Björkman 2017). Compared with the uterus, involution of the cervix is very rapid and completed within 1 week (Busch 2007, Kudlac & Groch 1979).

Resumption of Ovarian Function. Weaning sows immediately after parturition (zero-weaned sows) or <3 weeks postpartum results in a higher incidence of anoestrus and a longer weaning-to-oestrus interval, ovulation failure, or a reduced litter size (Peters et al. 1969, Elliot et al. 1980, Varley & Atkinson 1985, Soede et al. 2009). Sows that fail to ovulate either show follicular regression, with a short/intermittent oestrus, or develop ovarian cysts. If sows are weaned >3 weeks, 98% of them show oestrus signs within a normal weaning-to-oestrus interval of less than 7 days, of which 98% ovulate (Knox & Rodriguez Zas 2001). Anoestrus and cystic ovaries in sows weaned at <3 weeks may be due to the lack of a preovulatory LH surge. The lack of the LH surge is believed to be due to high levels of corticosteroids observed in early weaned sows (Ryan & Raeside 1991) or an impaired responsiveness to a positive feedback of estradiol-17β at the hypothalamic–pituitary level (Soede et al. 2011). The impaired responsiveness may be related to depleted LH stores just after farrowing that will progressively restore during lactation (Crighton & Lamming 1969, Bevers et al. 1981). LH concentrations and the number of LH pulses remain low during days 4 to 14 and gradually increase thereafter (Stevenson et al. 1981, Shaw & Foxcroft 1985, De Rensis et al. 1993b). Besides the restoration of LH pulsatilty, follicular development during early lactation is suppressed and characterised by a large population of small-sized follicles (< 3 mm) and a small population of medium-sized follicles (3–4 mm) (Britt 1985). During the second week postpartum the number of small-sized follicles increases, and during the third week the overall number of follicles and the number of medium-sized follicles increases (Busch 2007). Nevertheless, a preovulatory LH surge will not occur before weaning because suckling and piglet proximity stimulates opioid release (De Rensis et al. 1993b). These opioids inhibit the GnRH pulse generator through neuroendocrine reflexes and therefore the LH secretion. If and how uterine involution affects the restoration of LH concentrations and pulsatility, and therefore follicular dynamics at the ovary, is not known. The restoration of LH-pulsatility and the shift in the follicular pool occur around the same time that uterine involution is complete – during the third week postpartum. Thus it is possible that uterine involution plays a role in the restoration of LH pulsatility and therefore follicular activity. Other factors that affect the degree of LH suppression are the suckling intensity and the energy balance of the sow (Soede et al. 2011).

Postpartum Dysgalactia Syndrome, PDS. PDS is the major puerperal disease in pigs. It affects both the sow and neonatal piglets during their first 1 to 3 days of life. It is characterised by insufficient colostrum and milk production by the sow and, as consequence, results in reduced intake by the piglets during the first days of their lives.

Martineau et al. (1992) listed the symptoms that may be present in the sow and the piglets, resulting in reduced herd productivity. In the sow the common general symptoms include fever (> 40°C), loss of appetite, and lethargy. The local symptoms are observed as inflammation of the mammary glands, dysgalactia, and metritis. In piglets < 1 week old, there is increased mortality, diarrhoea, and increased heterogeneity in the litter, and for those more than 1 week old, there is increased heterogeneity of the litter and low weaning weights. For the herd there is a reduced number of piglets/sow/year. PDS is considered to be a multifactorial disease involving different pathways.

The current hypothesis is that interactions between endotoxin produced by Gram-negative bacteria in the gut, mammary gland and/or uterus, and alterations in the immune and endocrine functions, play a central role in the development of PDS (Maes et al. 2010, Martineau et al. 2012).

In the study by Bostedt (1998), 24.4% of gilts suffered from a feverish puerperal illness; 24.4% exclusively showed signs of mastitis; in 29.5% there was a combination of mastitis and an inflammatory infection of the genital tract; 46.1% of the cases had only infection of the reproductive tract. Predominantly E. coli, followed by Staphylococcus spp. and Streptococcus spp., were isolated from the genital tract. The prominent role of E. coli has also been shown in puerperal mastitis, and therefore E. coli is considered the causative pathogen for agalactia in the majority of cases (Gerjets & Kemper 2009). This is supported by a study of Nachreiner and Ginther (1974) that challenged periparturient sows with lipopolysaccharide (LPS) endotoxin of E. coli and in which sows generated symptoms similar to postpartum agalactia.

There is actually little information available about if, and how, farrowing affects PDS. There seems to be a connection between a prolonged duration of farrowing and PDS. For example, both have common risk factors. These are: (1) constipation (Peltoniemi & Oliviero 2015, Hermansson et al. 1978, Martineau et al. 1992, Oliviero et al. 2009); (2) increased back fat (Göransson et al. 1989. Oliviero et al. 2009); (3) no farrowing supervision (Papadopoulos et al. 2010, Björkman et al. unpublished); (4) restricted space in farrowing crates (Bäckstrom et al. 1984, Cariolet 1991, Oliviero et al. 2008); and (5) farrowing induction (Papadopoulos et al. 2010, Smith 1982). Furthermore, associations between prolonged farrowing and general signs of PDS (Tummaruk & Sang-Gassanee 2013, Bostedt et al. 1998), such as fever, anorexia, and metritis (Björkman 2017) have been demonstrated. There seems to be an effect of farrowing duration on puerperal diseases. In a study by Tummaruk and Sang-Gassanee (2013), the percentage of sows with fever during the first 24 hours postpartum increased from 40% to 100%, when the farrowing duration increased from <2 to 4 to 8 h. Furthermore, until the third day postpartum, the percentage of sows with reduced appetite was higher than in sows with a farrowing duration 4 to 8 hours than in sows with <4 hours. A study by Bostedt et al. (1998), which examined gilts suffering from a feverish puerperal illness, found that the farrowing duration had a significant effect. Of the sows, 85.9% of sows with consequent puerperal illness had farrowing durations of >6 hours, whereas

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78.8% of control sows completed parturition in <3 hours. Other significant factors affecting the incidence of feverish puerperal illness were the frequency of obstetrical intervention and the stillbirth rate. The frequency of obstetrical intervention measured in the group of sows was 27%, whereas it was 9.5% in the control group. The stillbirth rate was significant higher in the ailing group than in the control group. Björkman et al. (unpublished) have linked exactly the same factors with puerperal metritis. When the birth canal was palpated and/or piglets removed manually, the incidence of puerperal metritis increased. The same association was found with the number of stillborn piglets. Furthermore, retained retention of placentae was shown to increase the incidence of puerperal metritis significantly.

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18

Manipulative Delivery *per vaginum* in Dogs and Cats

GARY C. W. ENGLAND

Introduction

Faulty fetal disposition, together with a smaller number of cases of moderate fetal oversize, fetal malformation, and fetal death, account for approximately 25% of cases of dystocia in bitches and queens. There are breed differences in causes of dystocia (O'Neill et al. 2017), and in some breeds the morphological features of the fetus will certainly necessitate a Caesarean hysterotomy. Nevertheless, some cases of fetal dystocia may be amenable to vaginal manipulation and traction, depending on the cause where they obstruct within the birth canal. In some cases manipulation may be attempted as a first line before implementation of a Caesarean hysterotomy (Münnich & Küchenmeister 2009). There is, though, an important place for manipulative obstetrical procedures to treat dystocia, particularly simple ones that, when implemented, can be followed by normal expulsion of the remainder of the litter. Importantly, obstructive dystocia is frequently followed by secondary uterine inertia. Although this can be treated by the administration of oxytocin, if the number of fetuses yet to be delivered is large, this may require multiple doses and result in prolonged parturition, risking fetal death. In these cases, it may be simplest to revert to Caesarean operation to ensure the prompt delivery of the remaining fetuses.

For any cases of vaginal manipulation, it is essential to ensure appropriate restraint of the dam that may be anxious and difficult to examine. Attention should be paid to cleaning the vulva and to appropriate hand hygiene before conducting a digital examination. Copious lubrication with a water-soluble lubricant is helpful in cases of moderate fetal oversize or when the tract has become dry because of prolonged dystocia.

Digital Manipulations

As for evaluation of any parturient animal, it is best to assess the presentation, position, and posture of the fetus within the birth canal. This facilitates decision making about grasping the fetus, use of instruments, and direction of traction.

Before resorting to assistance using instruments, the use of fingers should be fully exploited because they are gentler and can be used more sensitively than instruments. This technique is valuable when parts of the fetus have passed through the pelvic inlet and are reliably palpated within the vagina. During all these manipulations, in small or medium-sized bitches, it is helpful to fix the position of the fetus in the uterus by gripping it with the other hand through the abdominal wall and to direct the fetus towards the pelvic inlet.

In these instances, if the fetus is in anterior presentation it is often possible to grasp it by inserting the index or middle finger over the occiput and placing the thumb into the intermaxillary space, or by hooking the index and middle fingers and placing them behind the head, thus trapping the neck (Fig. 18.1). This will be sufficient to allow redirection and traction (see later in this chapter). For fetuses in posterior presentation, the index and middle fingers can be used to hook cranially to the wings of the ilium and provide a firm grip on the fetal pelvis. For cases of breech presentation (posterior presentation with the limbs flexed cranially), it is generally possible to hook the fingers around the retained limbs and draw them upwards and backwards into the maternal pelvis.

Frequently, these manipulation result in eliciting Ferguson's reflex, such that there will be additional uterine contractions, supplemented by abdominal contractions.

Once parts of the fetus are protruding from the vulva, traction delivery is generally simple. For fetuses in normal anterior or posterior presentation with the fetus present within the vestibule, it is usually a relatively simple matter to insert the finger beneath the fetal chin or around the fetal pelvis and, by drawing it upwards, direct the fetus in line with the birth canal (Figs 18.2 and 18.3).

The Use of Instruments

When parts of the fetus have already entered the pelvic inlet and occupy the vagina, Hobday's vectis (see Fig. 18.8) is a useful instrument. The vectis is passed into the vagina and, according to the presentation, over the dorsal aspect of the fetal head or pelvis, and by pressure downwards is thus engaged behind the occiput or tuber coxae. The index finger is then introduced and pressed upwards into the intermaxillary space or in front of the fetal pelvis. In this manner the opposing forces of the vectis above and the finger beneath enable sufficient compression to 'trap' the fetus, allowing it to be grasped adequately for traction without injury (Fig. 18.4). The method may be successful in cases of anterior presentation in which the forelimbs are flexed caudally and cannot be corrected because they do not necessarily contribute substantially to the diameter of the fetus such that, with suitable lubrication, the fetus may be delivered with traction.

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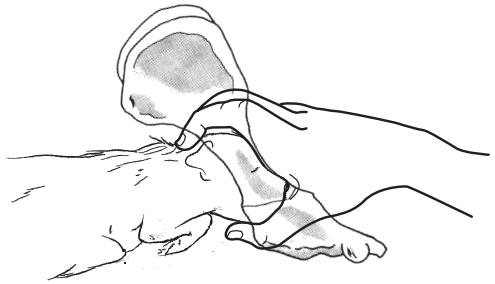
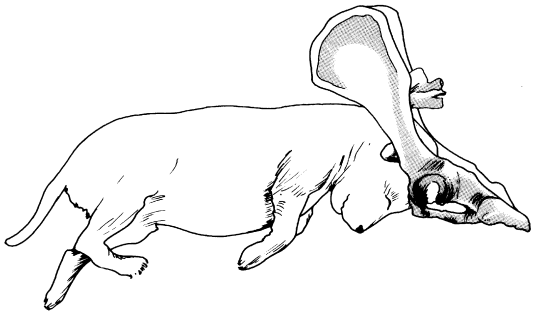
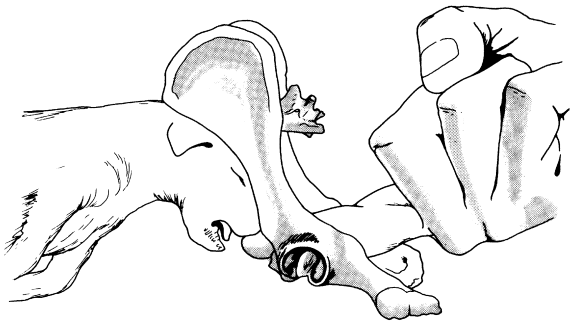


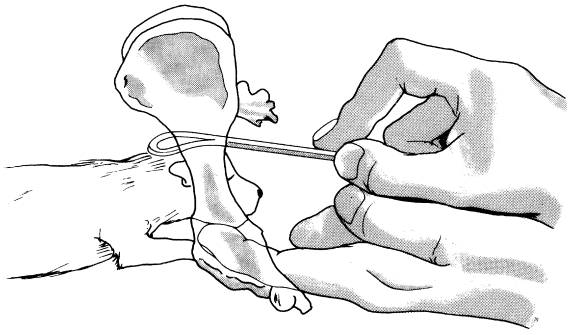
Fig. 18.1 Puppy is in normal disposition, but traction is applied by tightly grasping the head between the index and middle fingers.



• **Fig. 18.2** Vertex posture ('butt' presentation) with bilateral shoulder flexion.

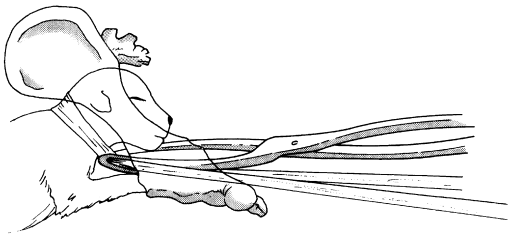


• **Fig. 18.3** Correction of the vertex posture with the finger.



• **Fig. 18.4** Traction applied to a puppy's head using the vectis and finger.

In cases of fetomaternal disproportion in anterior presentation, in which the fetus is entirely in the uterus and obstruction is caused by the size of the head, Roberts's snare forceps are of great value, particularly in small bitches and cats. Such cases may also be associated with retention of the forelimbs. Should the latter be the case, it is better to attempt delivery with the posture uncorrected, for the forelegs will cause no greater obstruction lying



• **Fig. 18.5** Roberts's snare forceps applied to the fetal neck.

alongside the chest than they would if extended; moreover, the subsequent traction, applied as it is to the head only, may cause the elbows alongside the head to become impacted at the pelvic inlet. Snare forceps are used as follows: The fetus is fixed at the pelvic brim by holding it through the abdominal wall; the closed forceps carrying the snare are passed into the uterus and over the fetal head until they lie above the neck. The jaws are then opened as widely as possible and depressed downwards until they lie ventral to the neck and are then closed. In this way, an encircling noose has been applied. By traction on the free ends of the snare, the noose is drawn tight, and it is held in position by the forceps. Traction is then applied to the forceps and the free ends of the snare (Fig. 18.5).

If snare forceps are unavailable, it is possible to use Rampley's sponge-holding forceps (Freak 1948). These provide a suitable grip, but obviously the ratchet mechanism should not be used. In this manner, traction can be applied to a living fetus similar to previously outlined. Using the index finger as a guide to their application, the forceps are lightly fixed across the lateral aspects of the head, or to the upper or lower jaw, or even the whole snout. In the case of a posterior presentation, they may be applied to the upper portion of a hindlimb until the fetal pelvis is drawn into the maternal inlet and then a more secure hold is obtained. It is best not to grasp distal parts of the limb because these are easily traumatised resulting in lifelong damage.

Lateral deviation of the head and limbs displaced dorsally over the neck (nape posture) are abnormalities that require special consideration. These initially may be difficult to diagnose and do require correction to allow delivery of the fetus. In lateral deviation of the head, the forelimb on the side opposite to the neck flexion has generally passed through the pelvic inlet (Fig. 18.6). Thus the presence of a single forelimb in the cranial vagina indicates a likelihood of the condition. To verify the diagnosis and also to ascertain the side to which the head is deviated, the fetus must first be repelled cranially. A finger is then used, directed laterally towards the iliac shaft in order to detect the fetal occiput or ears. In the small bitch or normal-size queen, this may not be difficult, but in the large animal the length of the maternal pelvis and of the fetal neck are often such that it is impossible to make an accurate diagnosis, let alone correct the condition. In a protracted case, it may be impossible to obtain the space in front of the pelvis necessary for exploration with the finger. Rampley's forceps can be used as both an aid to diagnosis and to the correction of downward and lateral deviation of the head (Freak 1948). Once the fetus is grasped, it may be raised sufficiently into the birth canal to allow a more complete examination with the finger, when fetal ears may be recognised lying just below the pelvic brim. Using the forceps to anchor and provide retropulsion to the fetus, the operator's other hand is used to apply transabdominal pressure to lift the fetus further into the birth canal. Correction has to be undertaken in stages, obtaining a new grip on the head after each repulsion. When the head is

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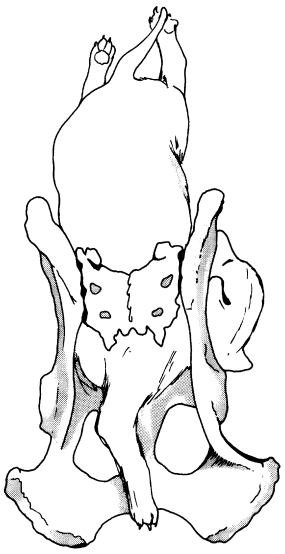


• **Fig. 18.6** Lateral deviation of the head (shoulder presentation).

displaced laterally, the forceps are used to repel the fetus diagonally away from the side to which the head is flexed. It may be helpful to position the dam in lateral recumbency, with the side to which the head is turned being uppermost. In this manner, the repulsion is directed downwards, and the weight of the fetal head helps in its repositioning. Again, correction may be undertaken in several stages, with assistance derived from transabdominal manipulation.

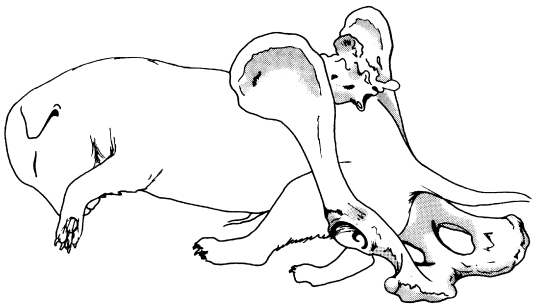
Delivery by Forced Traction

In most cases, traction is synchronised with the abdominal contractions of the dam, and the direction of traction follows a caudal and ventral direction to ensure minimal damage to the birth canal. In some cases, there may be absence of contractions from the dam, particularly in protracted cases of dystocia, with dead and emphysematous fetuses. Forceful traction should always be avoided in the case of a living fetus, for the grip of the forceps generally causes it severe injury. Hobday's forceps are generally employed.

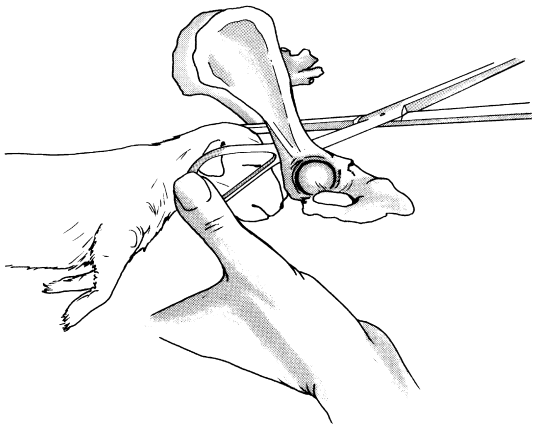
It should always be remembered that a Caesarean operation or, in the case of putrid fetuses, hysterectomy, will carry a better prognosis for the bitch or queen than prolonged attempts at forceps delivery.

In cases requiring traction, even when the fetus is dead, the presentation, position, and posture should be evaluated to enable correction before application of traction; this may necessitate initial repulsion (Fig. 18.7). The aim is to obtain a secure grip across the fetal cranium or pelvis, so that considerable traction can be applied. The application of the forceps to a limb or the lower or upper jaw is generally futile because the force that is necessary to be applied causes either the forceps to slip or the parts to be torn away.

Forced traction should be carried out under general anaesthesia and may be undertaken before a Caesarean if the dystocia affects only the last fetus. Forced traction is best undertaken with the dam in ventral recumbency. The position of the presented fetus is fixed by gripping it through the abdominal wall. The closed forceps are introduced into the vulva and directed at first upwards until they have reached the pelvic floor, then horizontally forwards through the pelvic canal, and finally slightly downwards and forwards into the uterus. Here, the fetal extremity will be felt and identified. The jaws of the forceps are now opened as widely as possible and again depressed downwards. On closing them, it becomes clear



• **Fig. 18.7** Bilateral hip flexion posture (breech presentation).



• **Fig. 18.8** Delivery of a puppy with retention of the forelimbs using Hobday's forceps. When the position of the fetus is fixed through the abdominal wall with the left hand, the forceps are applied to the skull with the right.

from the extent to which the handles are apart that the whole width of a fetal head or pelvis has been gripped. On no account should traction be applied until the operator is satisfied that he or she has a firm grip on the cranium or pelvis (Fig. 18.8). The operator is always fearful that vaginal wall has been trapped by the forceps in addition to the fetus. Fortunately, if the forceps are applied within the vagina as described previously, this is uncommon. Nevertheless, as soon as the secured part of the fetus has been retracted sufficiently caudally to a point that can be reached with a finger, the operator should ensure by palpation that the uterine wall is not trapped.

Steady traction is then applied, initially in a dorsal and caudal direction, to lift the fetus over the dam's pelvis into the birth canal. From this point, delivery is relatively easy. The direction of traction changes to a caudal and ventral direction to follow the anatomy of the birth canal. There is a limit to the amount of force that can safely be applied; severe traction may cause tearing of the vagina at the pelvic brim. In neglected cases in which the fetus is putrid, the application of traction may result in breakage of the fetus. Copious lubrication may help facilitate delivery and prevent breakage of the fetus.

After Delivery

After any cases of assisted vaginal delivery, the birth canal should be examined to ensure that there is no tearing or splitting of the vaginal or vestibular wall. Appropriate antimicrobial therapy is warranted in cases of manipulative delivery to reduce the risk of postpartum metritis.

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In cases of simple fetal dystocia corrected by vaginal manipulation, normal parturition may recommence. However, in many cases, secondary inertia will have ensued necessitating administration of oxytocin. Low dose oxytocin is most appropriate (0.04 IU/kg), administered every 30 minutes usually to a maximum of three doses.

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Caesarean Hysterotomy in Dogs and Cats

GARY C. W. ENGLAND

Introduction

There are multiple indications for Caesarean hysterotomy in the bitch and queen. These include obstructive dystocia, primary and secondary uterine inertia, and increasingly, at least in the bitch, as an elective procedure in which fetal oversize is predicted, commonly in brachycephalic breeds. As a result, there may be a wide variation in the status of the dam, from being substantially compromised to clinically normal. Anaesthetic regimes therefore should be planned to allow for this variation in status of the dam.

A significant complication to the assessment of bitches with potential dystocia is the large variability in 'apparent' pregnancy length; the interval between mating and the onset of parturition can vary from 58 to 72 days. This is despite the 'actual' (or endocrinological) length of pregnancy having a more limited variation of 63 ± 1 days from ovulation to the onset of parturition (Concannon et al. 1983). The difference between actual and apparent pregnancy length is primarily a result of the timing of mating that may occur many days before or many days after ovulation; the dog is unique in domesticated species in being able to achieve pregnancy over a longer time period because of significantly longer time of sperm survival in the female tract and prolonged viability of ovulated oocytes respectively. The large variability in apparent pregnancy length, especially pregnancies that appear to be very long (associated with mating before ovulation), creates the specific challenge of differentiating this normal pregnancy from primary uterine inertia (which is the most common cause of dystocia seen in the bitch). In the queen the length of pregnancy is less variable at a mean of 67 ± 3 days from mating to parturition, with a range of 62 to 71 days.

Pregnancy Length and Predicting Parturition

Understanding of normal pregnancy length, particularly in the bitch, is important especially to allow the differentiation of a normal apparently long pregnancy from primary uterine inertia, and also for cases when elective Caesarean is planned so that immature fetuses are not inadvertently delivered.

Normal pregnancy in the bitch and queen appears to be terminated, as in other species, by fetal signalling that ultimately results in a cascade of events resulting in prostaglandin release (see Chapter 6). Plasma progesterone concentrations therefore decline, and there is then an increase in uterine contractions and softening of the cervix. In bitches the onset of parturition occurs 63 ± 1 days from ovulation. Normal pregnancy may be slightly longer when fetal number (litter size) is small and also in some breeds (e.g., German Shepherd dog).

The anticipated day of parturition in the bitch can be predicted from assessment of the time of ovulation made during the breeding oestrus (Kutzler et al. 2003) (for example, parturition occurs approximately 61 days after progesterone exceeds 32 nmol/l), or by measurement of fetal size or stage of development at the time of ultrasound pregnancy diagnosis (England et al. 1990), or by monitoring the bitch in the last few days of pregnancy. In the queen, ultrasound assessment of fetal size and appearance may similarly allow the prediction of the onset of parturition, and some of the features of impending parturition are also comparable to those noted in the bitch.

For the bitch in particular the normal sequence of events seen in late pregnancy are that vulval and perineal tissues relax as a result of increasing relaxin concentrations and progesterone concentrations decline (termed the Stage of Preparation); as a result, there is a significant decrease in rectal temperature, the volume of mucus at the cervix increases, and the cervix begins to open (this can be seen endoscopically).

Subsequently uterine contractions commence (termed First Stage parturition) and may be seen by careful observation of the flank or by the measurement of uterine electrical activity. These contractions are often associated with anorexia, shivering, nesting, and vulval discharge; they are followed by the onset of abdominal contractions (termed Second Stage parturition). Generally, the sequence of events follows the time course noted in Table 19.1.

In some species there are changes in the ionic composition of milk that can be used to predict parturition, but this does not appear to be the case at least in the bitch (England & Verstegen 1997).

Indication for Caesarean Hysterotomy

The decision to perform a Caesarean operation is commonly undertaken in one of three particular circumstances:

A. Obstructive dystocia with or without secondary uterine inertia

Caesarean hysterotomy is likely in the bitch or queen as a result of a clear indication of an intractable dystocia after a digital examination of the vestibule and vagina, or when secondary uterine inertia follows the correction of dystocia and: (i) there is inadequate response to oxytocin administration; (ii) there is a large litter size such that parturition will inevitably be prolonged; or (iii) there are signs of fetal stress (see later in this chapter).

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TABLE 19.1 Normal time sequence of prepartum events in the bitch

Parameter	Relation to Parturition
Initial abrupt decline in progesterone	Approximately 36 hours before parturition
Rectal temperature declines to less than 37°C	No more than 36 hours before parturition
Progesterone concentration decreases to less than 2.0 ng/mL (6 nmol/l)	No more than 26 hours before parturition
Onset of uterine contractions (First Stage)	No more than 24 hours before parturition
Mucus at cervix increases and cervix opens	No more than 12 hours before parturition
Onset of abdominal contractions (Second Stage)	No more than 2 hours before parturition

B. Primary uterine inertia

Primary uterine inertia accounts for more than 70% of cases of dystocia seen in bitches and queens. In these, the common sequence of events is that progesterone concentrations decline (Stage of Preparation), uterine contractions begin (First Stage commences) but may be weak, and then uterine contractions cease. There are usually no abdominal contractions (no Second Stage). Unless the owner is experienced, the initial signs may be missed, and the owner is therefore unaware that parturition is overdue because of primary uterine inertia until placental separation commences (commonly indicated by the presence of a green/brown vulval discharge associated with release of pigment (uteroverdin) from the marginal haematoma of the placenta). Fetal death occurs rapidly at this point. When the clinical history is not informative and it is not clear whether the bitch has primary uterine inertia or parturition is not yet due, measurement of progesterone concentrations (England & Verstegen 1996) may be informative; basal progesterone indicates parturition is overdue or imminent.

If detected early before placental separation, cases may respond to exogenous oxytocin administration. Many cases of primary uterine inertia are, however, associated with either a small litter size, large litter size, or a dam that is obese, aged, or debilitated. In these cases, undertaking Caesarean hysterotomy may be more appropriate than attempting oxytocin administration which may not be successful.

C. Elective Caesarean hysterotomy

Elective Caesarean surgery may be considered when there is expected fetomaternal disproportion, perhaps most commonly in dogs of the brachycephalic breed or queens in which there is narrowed birth canal as a result of a previously fractured pelvis. In these cases the challenge is identification of the optimal time of surgery, which can be difficult to predict if only the date of mating is known because of the large variation in apparent pregnancy length as described previously. Different approaches are taken to decide on which day to perform an elective Caesarean, with some practitioners waiting until the onset of clinical signs of the onset of First or Second Stage parturition, others relying on detection of the prepartum decrease in rectal temperature, and others monitoring the decline in plasma progesterone concentration.

Whenever the late pregnant bitch is examined, the key feature is always to consider whether there is a failure of the normal events of parturition to progress. This requires knowledge of the normal time sequence of events, which may be modified depending on the age and parity of the dam as well as the litter size. Measurement of plasma progesterone is a useful indicator of the stage of parturition (Table 19.1), but for accurate assessment repeated samples are required (Rota et al. 2015). Additional critical signs include the appearance of a green/brown/red coloured vulval discharge that is indicative of placental separation. The presence of placental separation may modify the clinical decision taken about a given case. Furthermore, ongoing monitoring of the fetus during late pregnancy and parturition may include attempts to detect fetal stress by assessment of fetal heart rate. Normal fetal heart rate at term is between 170 to 230 bpm (or 3–4 times maternal heart rate). The immediate response of a hypoxic fetus is a short-term increase in heart rate; however, the most common finding in veterinary practice is chronic fetal hypoxia detected by a decrease in fetal heart rate. Fetal heart rates less than 150 bpm indicate hypoxia (fetuses are stressed); fetal heart rates less than 130 bpm are associated with poor survival if delivery does not occur within 2 to 3 hours; fetal heart rates less than 100 bpm generally require immediate intervention to hasten delivery before demise of the fetuses.

Anaesthetic Considerations

Substantial physiological changes occur in late pregnancy. These include a reduction in functional residual capacity, and in the bitch, a substantial normochromic normocytic anaemia becomes maximal near to term and can involve a reduction of haematocrit to 30%. Pregnant animals are at risk of hypoxaemia and preoxygenation before induction of anaesthesia is indicated. Animals may also be debilitated and dehydrated if presented because of dystocia, and in these cases intravenous fluid therapy is mandatory.

It is not within the scope of this text to review anaesthetic regimes for the Caesarean hysterotomy; suffice to say, they should take account of the clinical presentation of the dam. A recent review of appropriate analgesia and anaesthesia regimes has been published by the WSAVA Global Pain Treatise and can be found at www.wsava.org.

Operative Technique

The surgical procedure is similar for both bitches and queens, although many variations in technique have been described (Traas 2008). It is common practice to undertake preparatory activities such as hair clipping and initial antiseptic scrub before induction of anaesthesia.

The female is placed into dorsal recumbency on a slightly tilted table such that the dam's head is highest as this may reduce abdominal pressure on the diaphragm. The surgical site is prepared and draped from the xiphoid to the pubis, and a ventral midline skin incision is made from just cranial to the umbilicus to just cranial to the pubis. Electrocautery may be useful to control skin and subcutaneous bleeding from vessels that may be enlarged associated with mammary development. The abdomen is opened in a routine manner to avoid possible penetration of the distended uterus lying immediately beneath. The common procedure is to carefully lift the gravid uterus from the abdominal cavity, ensuring no tearing of the uterus or of the broad ligament vessels. Moistened swabs can help support the uterus, prevent it from drying, and avoid contamination of the peritoneal cavity with allantoic or amniotic fluid. The number of fetuses present, the degree of coiling of the uterus, and the innate pressure within the uterus will influence

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whether the dorsal or ventral surface of the uterus is uppermost when exteriorised. Once exteriorised, it is likely that uterine blood flow is compromised, and so removal of the fetuses should be undertaken rapidly. If possible, a relatively avascular area of the uterine body is chosen for the initial hysterotomy incision. The incision is best made by lifting the uterine wall away from the fetus, making a small stab incision with a scapula and extending the incision in a longitudinal manner with scissors. The incision should be sufficiently long to allow manipulation and delivery of the fetus without risk of tearing of the uterus. The adjacent fetus is located, grasped by the surgeon through the fetal membranes, and extracted through the uterine incision. At this stage no attention needs to be paid to specific fetal membranes as they will be ruptured during removal of the fetus. Forceps are applied to the umbilicus, and the fetus is passed to an assistant to revive. Gentle traction should be applied to the remaining umbilical cord, and the placenta should be removed if it detaches easily from the uterine wall; otherwise it can be left in place. Remaining fetuses are manipulated by gently squeezing them to the hysterotomy incision and are removed in a similar way. In large breed bitches with many fetuses, it may be necessary to make secondary incisions within both uterine horns to facilitate rapid removal of all pups. The entire length of the uterus should be examined to ensure that no fetuses remain and special attention should be paid to the distal uterine body/vagina. Once all fetuses have been removed, the uterus normally starts to decrease in size quite rapidly. The hysterotomy incision is closed with a monofilament absorbent suture material with a continuous pattern using either a single inverting pattern or an initial appositional pattern followed by a second inverting pattern. After closure of the hysterotomy incision, if the uterus has not started to involute then it is appropriate to administer exogenous oxytocin at 0.04 IU/kg. The uterus is lavaged with saline, inspected for any tears to the uterine wall or broad ligament, and then replaced into the abdominal cavity. If the abdominal cavity has been contaminated, abdominal lavage should also be performed. Closure of the abdominal wall is routine except that many veterinary surgeons place subcuticular sutures to minimise interference from the neonates. Placentae that were not removed through the hysterotomy incision are normally expulsed over the first 48 hours after surgery. Bitches with retained placentae should be monitored to ensure that metritis does not develop.

In a small proportion of cases in which dystocia has been long-standing and especially if there are emphysematous fetuses, the uterus may be devitalised. Ovariohysterectomy may be required and is best performed with the fetuses in situ. This technique of en bloc ovariohysterectomy may also be performed when the fetuses are alive after a presurgical decision to perform ovariohysterectomy. The technique follows a similar approach with exteriorisation of the uterus as described previously. The broad ligament vessels are ligated and the broad ligament is transected. If a fetus is present within the uterine body, it is pushed cranially away from the cervix, and two pairs of forceps are placed across the uterine body. Two pairs of forceps are similarly placed across each ovarian pedicle. Transection between the pairs of forceps allows the uterus to be removed and passed to an assistant who removes and revives the neonates. The surgeon then ligates the uterine and ovarian stumps in the normal manner. This technique is not associated with reduced milk production from the dam.

Resuscitation of Neonates

With modern anaesthetic regimes, neonates are often not significantly depressed at the time of removal. However, they have not been stimulated by passage through the birth canal, and there has been no time for amniotic fluid to drain from the respiratory tract. The airway should therefore be cleared of fluid using a towel or suction, the chest should be dried with a towel, and the neonate assessed for respiratory and cardiac activity (ABC; airway, breathing, circulation). If spontaneous breathing is not present, then artificial respiration should be initiated. Transtracheal intubation can be particularly useful as a short-term measure. Similarly, cardiac massage should be initiated if heart beats are absent. Once each neonate is stable, it can be transferred to a warm environment to recover while the surgery is completed. After surgery the dam's nipples should be cleaned to ensure no residual antiseptic (which may deter neonates from sucking) before neonates are encouraged to suck. Milk let-down can be encouraged with exogenous oxytocin at 0.04 IU/kg. In dams with inadequate milk production, prolactin agonists such as metoclopramide (0.1–0.2 mg/kg given three or four times daily for 5 days) can be administered.

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Injuries and Diseases Consequent Upon Parturition

TIMOTHY J. PARKINSON AND DAVID E. NOAKES

Trauma to the Birth Canal and Neighbouring Structures

There are numerous accidents and diseases that accompany or follow parturition. Some, such as prolapse of the uterus, retention of the fetal membranes, and puerperal infection, occur after the delivery of the fetus; the last two are considered elsewhere in the book. Traumatic lesions of the soft tissues of the genital tract or bony pelvis may lead to fatal haemorrhage or infection or to disability due to fractures, dislocations, or paralysis. Other complications of parturition include displacement, herniation, and rupture of the pelvic or abdominal organs. Parturition and the puerperium may also be complicated by metabolic diseases, particularly hypocalcaemia and, in adult cows, by displacement of the abomasum. A difficult foaling may be followed by laminitis or tetanus, and postpartum animals of all species may incur embolic pneumonia, toxaemia, septicaemia, and pyaemia as sequels to uterine infection. Endocarditis, unthriftiness, and sterility are possible later sequelae.

Although spontaneous injury may occur in unassisted deliveries, they are rare. The most frequent cause of parturient and postparturient disease is delay in giving obstetrical aid to dystocia cases, or bad and unsympathetic obstetrical practice, frequently by unskilled individuals. For this reason it is very important for the veterinarian attending dystocias to determine whether, and what, lay assistance has been given, both by questioning the person responsible for the care and by clinical examination of the genital tract. If skilled attention were to be provided at the correct time to animals with dystocia, the frequency and severity of postparturient disease would be markedly reduced.

Postpartum Haemorrhage

Bleeding from the maternal side of the placenta after natural separation of the placenta is only likely in carnivora, in which breakdown of the marginal haematoma is accompanied by a green or brown discharge of altered blood. If, however, premature dehiscence occurs when the afterbirth is removed during elective Caesarean hysterotomy, severe and even fatal haemorrhage may follow. In veterinary obstetrics the usual cause of serious haemorrhage is trauma of a uterine blood vessel by a fetal appendage, the application of forced traction before the cervix has fully dilated, or via an obstetrical instrument, of the hand of the obstetrician. After removal of the fetus, much blood may accumulate in the uterus before it begins to escape via the vagina; alternatively, blood may drain through a tear in the uterine wall into the abdomen.

When, after delivery of the offspring, there is a profuse haemorrhage from the vulva, the most likely source is the broken ends of the vessels of the umbilical cord which have recoiled into the vagina. This is likely to occur in uterine inertia in which, owing to poor uterine contractions, much of the blood from the fetal side of the placenta (allantochorion) is not expelled into the fetus during second stage labour. Similar bleeding from mares is seen when there is manual interference by unnecessary traction on the fetus resulting in premature rupture of the umbilicus; if this occurs, ligation of the cord as close to the fetal abdomen should be attempted. Such haemorrhage from the allantochorion does not affect the dam, but the neonate is thereby deprived of placental blood transfer, which can be a significant factor in causing cerebral anoxia in newborn foals (MacLeay 2000).

If profuse haemorrhage is occurring from a uterine laceration, prompt contraction of the uterus should be promoted by means of an injection of oxytocin. Next day, any blood clots should be manually removed. Haemorrhage associated with uterine rupture is managed when the uterine tear is repaired. When severe haemorrhage is occurring from a ruptured vaginal vessel, an attempt must be made to close the vessel. Ligation is usually not practicable, but artery forceps may be applied and left on for 24 hours. Where the vessel cannot be secured, an intravaginal pressure pack can be improvised with a large clean towel or by the insertion of a large role of surgical gauze or cotton wool. General symptoms of severe haemorrhage and shock can be counteracted by blood transfusion (4–5 litres) from a neighbouring animal or the use of blood volume expanders.

Fatal haemorrhage from vessels in the broad ligament can occur in the mare and cow. Ueno et al. (2010) reported 31 cases of fatal haemorrhage into the broad ligament of mares. Thirty of these had had over eight pregnancies and were over 10 years of age. The most common site of haemorrhage was the uterine artery (24 cases) and the internal pudendal artery (5 cases). They found that the smooth muscle of tunica media adjacent to the arterial ruptures showed atrophy and that there was fibrotic change of the tunica intima, together with disruption and/or calcification of the internal elastic lamina and proliferation of collagenous and elastic

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stroma. These lesions were most prominent in the uterine arteries of aged and multiparous mare, and were rare in mares that had not experienced such haemorrhage. The lumen of the proximal uterine artery of aged mares was sometimes dilated, forming small aneurysms. Reduced density of smooth muscle cells and fibrosis was present in the expanded wall of the aneurysm. Rooney (1964) recorded 10 cases of fatal haemorrhage in mares with similar pathology of the blood vessels. Ueno et al. (2010) considered that the most likely cause was age-related haemodynamic stress, although copper deficiency has also been suggested (McGladdery 2001). In mares the haemorrhage is sometimes contained within the broad ligament, with the formation of a large haematoma, which can be diagnosed by careful transrectal ultrasonography as a hyperechoic structure in the broad ligament. Attempts at surgical repair are rarely successful because stress-induced hypertension may exacerbate the problem (McGladdery 2001). A more cautious approach is preferable, such as sedation and the use of blood transfusions and blood volume expanders, with regular monitoring of blood values.

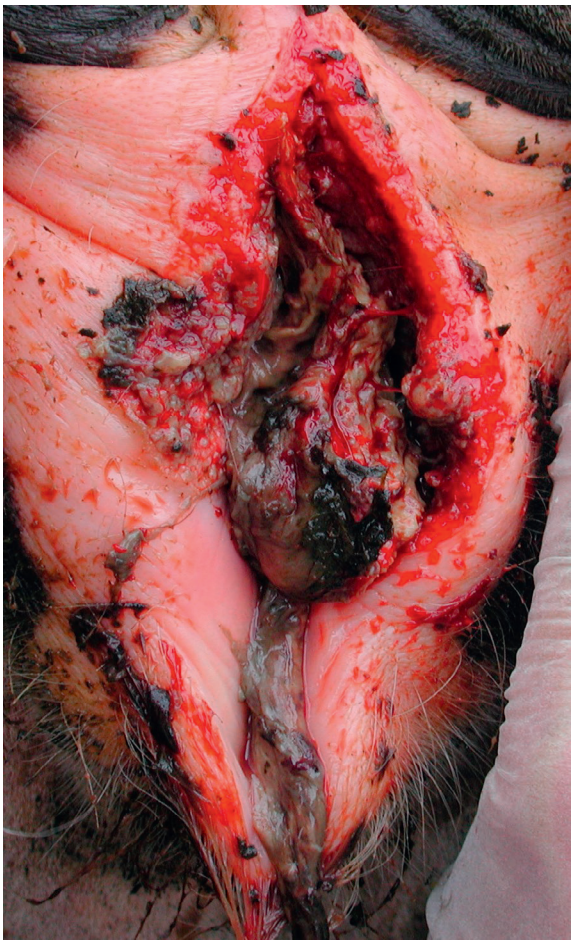
Lacerations and Contusions

Any part of the birth canal may suffer contusion during forcible extraction of the fetus, but the cervix and vulva are more likely to be lacerated than the dilatable vagina. The retroperitoneal fat surrounding the vagina of heifers of the beef breeds makes such animals particularly prone to vaginal contusion when the fetus is oversized. Infection with *Fusobacterium necrophorum* is then probable, and a severe necrotic vaginitis ensues. The condition is very painful and causes continuous, exhausting straining and marked toxaemia (Fig. 20.1). Pyogenic infection is also possible. All vaginal contusions and lacerations should be treated with mild emollient and antibiotic preparations; parenteral antibiotics should also be given. Caudal epidural anaesthesia, particularly when xylazine is used, gives temporary relief from straining (see Chapter 14). Lacerations, however modest in severity, can be a sign of more severe uterine disease or may predispose to it; in addition, they may have an effect on the return of subsequent ovarian cyclicity. Vieira-Neto et al. (2016) found that Holstein cows that had injuries to the vulva or caudal vagina 4–8 days post partum were more likely than normal cows to develop endometritis by 32 days. They were also slower to resume oestrous cycles and were less likely to conceive to their first insemination.

Rupture of the vagina should be repaired, if possible, by suturing, although access can be difficult. Infection after rupture may give rise to peritonitis, to severe cellulitis (which, if severe, can be accompanied by marked toxaemia and straining), or to abscess formation with subsequent vaginal constriction. Lacerations of the cervix may be sutured by applying vulsellum retraction forceps to the organ and withdrawing it to the vulva.

Wounds to the vulva and perineum are generally easily sutured, with any devitalised tissue, including any loosely attached portions of fat, being first removed. If lacerations of the vulva and perineum are left unsutured, scar tissue formation and distortion may impair the sphincter action of the vulva, with consequent aspiration of air, vaginitis, and metritis; a special, and much more difficult, operation is then required. When Caslick's operation to prevent vaginal aspiration has previously been performed in the mare (see Chapter 31), it should be resutured immediately after delivery. Repair of the vulva, perineum, and cervix may be conveniently carried out under caudal epidural anaesthesia.

In cows previously unsuspected organising haematomata of the vagina may suddenly prolapse from the vulva 4 to 6 weeks after parturition. These lesions resemble fibromata but are not neoplastic and are easily excised.



• **Fig. 20.1** Necrotic vaginitis, as a sequel to trauma of the birth canal during calving. (Courtesy Dr. Jakob Malmo.)

Haematoma of the Vulva

This is a sequel to contusion of the submucous tissue during delivery. One lip of the vulva is usually affected, and an obvious round swelling occupies the vulva orifice (Fig. 20.2). The condition may arise spontaneously in the mare, but in both cows and mares it is likely to follow assisted delivery in which considerable manipulation or forced traction was required. Haematoma of the vulva may be confused with prolapse, tumour, or cyst of the vagina. If left untreated, natural resolution usually occurs within a few weeks with resorption of fluid and regression of swelling; occasionally, pyogenic infection occurs and may be accompanied by fibrosis and distortion of the vulva with vaginal aspiration. If left for 3 or 4 days after labour, the haematoma may be safely incised and the clot removed without recurrence of haemorrhage. An abscess should be opened and drained.

Perineal Injuries at Parturition

Serious perineal injuries occur during the second stage of labour in both the cow and the mare, mostly in primiparous animals,

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• **Fig. 20.2** Vulva haematoma in the left labium.

and are often associated with poor relaxation of the vulva and perineum and/or unsympathetic forced traction. These injuries may be classified as first, second and third degree tears, and rectovaginal fistulae. Many heifers sustain slight superficial tearing of the upper commissure or other part of the vulva (first degree laceration) because of stretching during expulsion of the fetus; such lesions may heal satisfactorily by first intention without suturing, although it is preferable to do so whenever possible. Tears which extend more deeply into the perineum involving muscle (second degree laceration) destroy the sphincter effect of the vulva and lead to aspiration of air into the vagina, even though the integrity of the anus is not impaired. These require immediate surgical repair. With greater stretching and tearing during the second stage of labour, the wound may extend into and destroy the anal sphincter, thus creating a cloaca through which faeces fall into the caudal-most part of the vagina (third degree laceration) (Figs 20.3A and 20.4). Despite rapid epithelialisation, the abnormal communication between the terminal rectum and vagina persists, although its extent may be considerably reduced by wound granulation; complete surgical repair about 6 weeks after it occurred is required. In cattle, simple rectovaginal fistulae (Figs 20.5 and 20.6) without damage to the anal sphincter are uncommon spontaneous obstetrical injuries, although they occur as developmental anomalies in cases of anal atresia. They may also result from an unsuccessful attempted closure of a third degree perineal laceration in the mare.

In the mare the mechanism of perineal tearing is different. In this species, the initial injury is usually perforation of the roof of the vagina by a fetal forelimb, which may be pushed or reflected dorsally during the second stage of labour. As a result of sustained vigorous straining, the limb is then likely to perforate the rectum and be forced, possibly with the fetal head, through the anus, which in turn may be ruptured. Early recognition of the injury may allow repositioning of the fetal extremities and normal vaginal delivery, but deliberate incision of the perineum and anal sphincter is usually expedient if the rectum is already perforated, because a third degree defect is easier to repair surgically than a rectovaginal fistula, which would otherwise result. Mares in which a Caslick's closure of the upper vulval commissure has not been not reopened before foaling may sustain a similar injury in a slightly different way, with the tear extending dorsally from the vulva, as in cows. Records of cases presented for repair indicate that third degree tears are the most common perineal injury in the mare, but rectovaginal fistula formation is still more frequent in the mare than the cow. By contrast, second degree defects are rare in the mare but not uncommon in cows, simply because of the different mechanisms of tearing in the two species.

Perineal defects granulate and are epithelialised rapidly, but they are lacerated wounds with considerable tissue damage, and a degree of superficial sloughing is usual before granulation begins. The extent of inevitable tissue necrosis prejudices the likelihood of first intention healing after immediate suturing. It is nevertheless advisable to stitch deep perineal wounds that have not perforated the anal sphincter as soon as possible. Third degree tears with destruction of the sphincter and rectovaginal fistulae should be left to heal by granulation and surgical reconstruction can be undertaken later if necessary. The extent of such defects is considerably reduced by cicatrisation, and occasionally small, oblique fistulae in the mare close completely; however, in most cases a significant defect remains.

The clinical effects of a third degree defect are twofold: continuous aspiration of air into the vagina and contamination of the vaginal lumen with faecal fluids or, worse still, accumulation of faecal boluses in its terminal segment. Pneumovagina, in turn, by distorting the lumen, may lead to pooling of urine cranial to the external urethral meatus. Inevitably in both the cow and the mare, these factors result in gross bacterial contamination and ascending infection in the genital tract. In both species, therefore, sizeable cloacal lesions result in infertility, and affected mares are also aesthetically unsuited for other uses because of perineal incompetence. In cases of rectovaginal fistula, the degree of faecal contamination of the vagina depends on the extent of the fistula. The few animals that are able to maintain a normal pregnancy are generally found to have a caudally sited lesion of very small diameter.

Surgical intervention should be delayed until all tissue surfaces are covered by epithelium, which usually takes 6 weeks or so. In the mare the urinary bladder is sometimes everted soon after the injury occurs (Fig. 20.7), but it is easily replaced and retained if necessary with sutures. There is no need for other treatment during the intervening period except perhaps for tetanus prophylaxis.

Second degree defects can be satisfactorily obliterated by stripping the vaginal mucosa from the normal level of the upper vulval commissure dorsally on both sides and suturing the submucosal tissues as in a Caslick's operation.

One step and two step procedures for surgical reconstruction of the perineum were described by Götze (1938) and Aanes (1964), respectively. The main method in current use is a one-step procedure described by Stickle et al. (1979). The procedure is suitable for the cow or the mare: in the cow it should be performed under caudal epidural anaesthetic, and it is best to do so in the mare, although it

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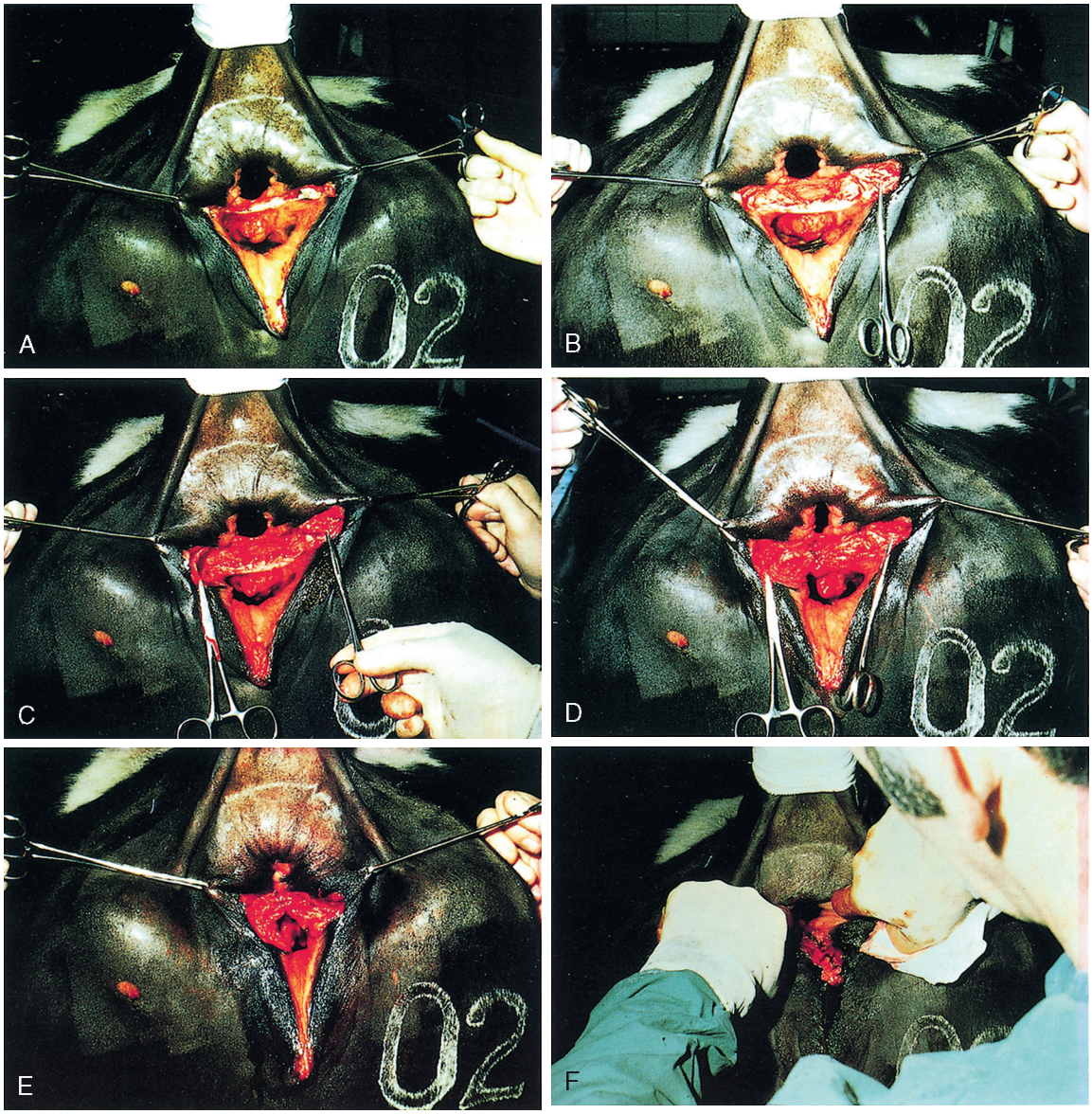

• **Fig. 20.3** Repair of third degree perineal laceration in a Friesian heifer. (A) The perineal laceration is exposed with cow under caudal epidural anaesthesia. (B) Commencement of the dissection of the vaginal mucosa. (C) Completion of the dissection of the vaginal mucosa; note exposed tissue ready for suturing. (D) Commencement of closure. (E) Closure almost completed. (F) Restoration of a complete shelf of tissue between rectum and vagina; the dorsal commissure of the vulva will require further repair.

can be performed under general anaesthesia. Cows require no dietary preparation, but, in the mare, a laxative diet without roughage is advisable for 3 days beforehand, followed by overnight starvation. After proper cleansing of the site, the rectum is gently packed with towelling; if the mare is anaesthetised, the bladder is catheterised to divert urine from the operation site. In cows the defect is usually no more than 6 cm deep from the perineum, but in the mare it can be considerably longer and sometimes extends almost to the cervix. In both species tissue forceps are placed on the cutaneous borders of the cloaca down to the normal level of the upper vulval commissure and on the caudal edge of the residual shelf. Bridges of skin across the defect are removed, and it is then possible to see a sharp demarcation between the vaginal and rectal mucosae (see Fig. 20.3A–C). The first stage of the procedure is to separate the vaginal mucous membrane from the tissues which will subsequently be apposed to create a shelf. The dissection begins at the level of the normal upper commissure and is extended dorsally on the mucocutaneous border and then cranially on both sides along the junction of vaginal and rectal mucous membranes until the incisions meet on the caudal edge of the residual shelf. The final stage of dissection is the separation of vaginal mucosa for 4 cm cranial to the edge of the shelf (see Fig. 20.3B and C). It is essential that all the vaginal mucous membrane is removed from the tissues that are to be sutured. There is minimal haemorrhage during the procedure and no need for haemostasis. In some cases, cicatrisation results in considerable asymmetry of the cloaca which should be corrected before suturing is begun. The curtain of separated vaginal mucosa is then included in a purse-ring type of suture.

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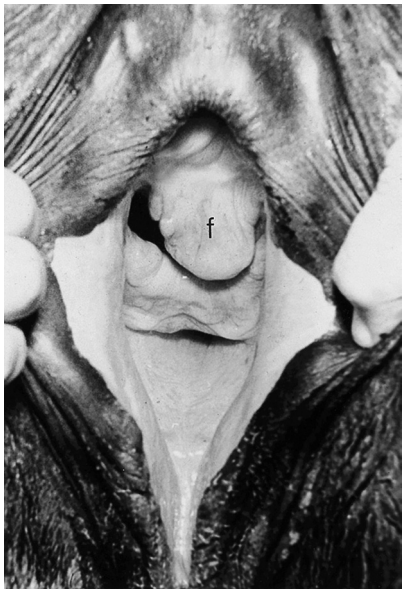
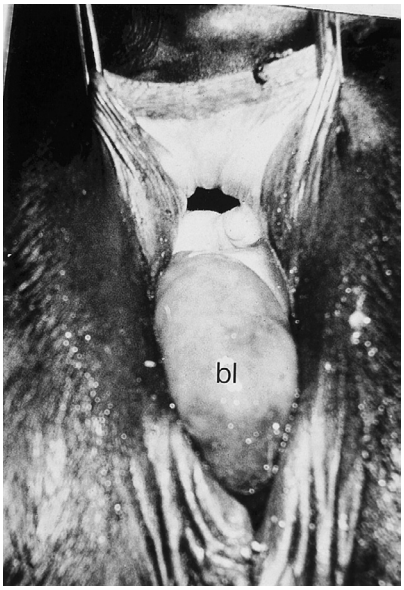


• **Fig. 20.4** Third degree perineal defect in the mare, showing a flap of mucosa (f) attached to the roof of the vagina at the caudal border of the residual shelf.



• **Fig. 20.7** Third degree perineal laceration in a mare with eversion of the bladder (bl).

• **Fig. 20.5** Acquired rectovaginal fistula in a cow. Vulva dilated to vaginal opening to fistula.

• **Fig. 20.6** Acquired rectovaginal fistula in a cow, with a bandage passed through the fistula.

An absorbable suture material is used: the sutures are placed and tied serially from the depth of the wound outwards (see Fig. 20.3D and E). The method of suturing is illustrated in Fig. 20.8.

It is most important that the stitches tighten properly, because dead space predisposes to wound breakdown. The operation is completed with mattress sutures in the perineal skin (Figs 20.3F and 20.9). Further minor closure of the upper commissure may be necessary under local anaesthetic infiltration when the integrity of the repair has been properly tested a month or so later. It should be emphasised that, although this operation restores breeding ability, it does not prevent air movement through the incompetent anal sphincter, a consideration which may be important in mares that are to be used for other purposes. In such animals a second operation to strengthen the sphincter can be attempted later by stripping mucocutaneous tissues in the defective segment and suturing whatever muscle remnants can be identified. The horse's anus is normally somewhat lax, and minor incompetence is no great detriment. If attempted reconstruction is unsuccessful, the operation can be repeated, but the prognosis is then less good because of local fibrosis and reduced vascularity. Unless the vulval length is inadvertently shortened during reconstruction, subsequent parturition in both the cow and the mare usually occurs normally without the risk of vulval tearing or the need for episiotomy.

Paradoxically, a simple rectovaginal fistula is more difficult to repair than a third degree defect. Aanes (1964) recommended that such lesions should first be converted into a cloaca (as in a third degree laceration) and repaired as such after granulation stops. The deliberate destruction of perineum and anal sphincter can be avoided by adopting a different surgical approach to such lesions. Unless the fistula is deeply sited, it can be exposed satisfactorily by a dorsal commissure episiotomy that is extended cranially under the anal sphincter and rectal floor beyond the fistula (Fig. 20.10). The rectal mucous membrane lining the lesion can then be securely inverted with sutures placed in a transverse direction in the submucosal tissues before the episiotomy is repaired in the

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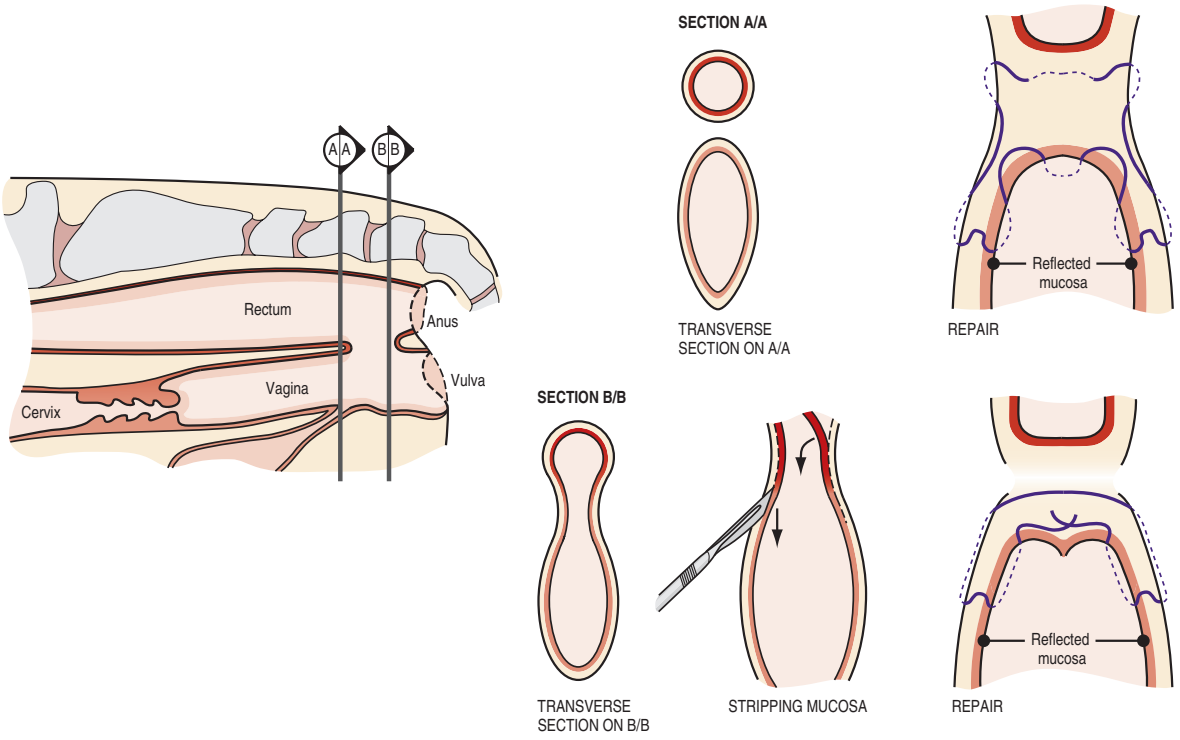

• **Fig. 20.8** Suturing technique for reconstruction of the perineum. (A) Below the shelf. (B) Caudal to the shelf.

• **Fig. 20.9** Completed one stage reconstruction of the perineum in a Friesian cow.

• **Fig. 20.10** A congenital rectovaginal fistula in a donkey exposed by episiotomy.

conventional way. Perineal defects are usually obvious in mares but are nevertheless sometimes not noticed by unwary purchasers. They are less obvious in cattle, particularly if the anal orifice remains intact.

Rupture of the Uterus or Vagina

Rupture of the uterus may occur spontaneously, but faulty obstetrical technique is a more frequent cause. Spontaneous rupture is most likely to arise in association with uterine torsion or with cervical nondilation, but is also possibly due to the gross uterine distension that occurs with twins in one horn, with hydrallantois, or with excessive fetal size. The most likely time of spontaneous rupture is in late gestation or during labour. Hopkins and Amor (1964)

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remarked on the association of spontaneous uterine rupture and breech presentation; they encountered three cases and cited four other cases from the literature. The most common site of rupture was the dorsal aspect of the left uterine horn, with the tear extending caudally to involve the uterine body and cervix. Their hypothesis is that breech presentation predisposes to rupture because the breech of the calf fully occupies the maternal pelvic inlet and allows no egress for the fetal fluid when the uterine and abdominal contractions build up the hydrostatic pressure within the uterus. In a review of 26 cases of uterine rupture, 18 of which were heifers, Pearson and Denny (1975) considered uterine torsion and fetomaternal disproportion to be major predisposing factors. In that series, 14 of the 26 fetuses were mainly or entirely within the peritoneal cavity; four were still alive at the time of laparotomy. According to the size of the rupture – which may heal without incident or allow escape of the conceptus in the abdomen – and to whether or not infection occurs, there is great variation in the syndrome; from cases in which no symptoms are shown to others in which shock and fatal toxaemia soon supervene. Thus in some instances the owner is unaware of the accident, and the only evidence of it is the subsequent finding of a uterine adhesion or of a mummified fetus among the abdominal viscera – so-called extrauterine or pseudoectopic pregnancy. When rupture occurs during labour and the fetus passes into the abdomen, labour pains and straining cease and uterine inertia may be suspected until a uterine exploration proves otherwise. Alternatively, the dam's intestines may prolapse into the uterus and even protrude from the vulva; the condition may then be confused with dystocia due to schistosoma reflexus (see Fig. 14.37) in visceral presentation.

Accidental rupture of the uterus is likely to occur in the most difficult dystocia cases: those in which the initial disposition of the fetus is markedly irregular and difficult to rectify, and those in which there has been much delay in treatment with the development of unfavourable complications. Insufficient uterine space for the extension of a limb or head, inordinate traction on a wrongly disposed or oversized fetus, and excessively vigorous retropulsion are the immediate causes of uterine rupture. When the cervix is incompletely dilated, traction on the fetus may cause rupture of that organ. Careless use of the obstetrical forceps in the bitch is a potential cause of uterine rupture. Lastly, rupture of the uterus may be due to external violence as, for example, when the parturient dam falls heavily or receives a severe kick or horn-gore on its abdomen.

When making the initial examination of a dystocia case, the veterinarian should always explore the genital tract for traumatic lesions that may have been caused by unskilled lay interference or that, rarely, may have arisen spontaneously. If uterine rupture is found then or if it occurs during subsequent manipulations, the obstetrician must decide – largely on considerations of size and site of the lesion and the amount of manipulation or traction still required to effect delivery – whether to proceed with the delivery *per vaginam* or whether to perform a Caesarean hysterotomy and repair the uterine rupture from the laparotomy site after the removal of the fetus. Except where a small dorsal rupture is discovered and the amount of obstetrical interference still required is small, a laparotomy is indicated. The procedure then adopted is almost identical to that described for a Caesarean hysterotomy, the only complication being the difficulties of accessing the site of uterine rupture from the abdominal incision. The accidental rupture may be extended and the fetus extracted, or if the tear is difficult to access, another surgical incision must be made for delivery, and then both it and the rupture must be repaired. The tear in the uterus is much more accessible for suturing after the fetus has been removed.

Spontaneous rupture of the vaginal wall in late pregnant ewes was first described by White (1961); since then, it has been reported by others (e.g., Knottenbelt 1988, Mosdol 1999). The small intestine passes into the vagina and protrudes from the vulva; frequently the ewe will be found dead. The precise aetiology of the disorder is still unknown. Initially, it was generally believed to be associated with cervical vaginal prolapse (CVP), although Mosdol (1999) found evidence that uterine torsion during late pregnancy might be involved. In one case, which was considered to have a similar aetiology, Fox (1962) noted complete prolapse of the intact pregnant uterus through a tear in the dorsal wall of the vagina. O'Neill (1961) observed several parturient ewes that were unable to lamb in which rupture of the uterus was present. Prompt Caesarean hysterotomy and repair of the uterine tear gave good results.

Prolapse of the Bladder

Prolapse of the bladder through the urethra is most likely in the mare (see also Chapter 13). In this species the urethral opening is wide and parturient straining very forceful. The organ becomes everted during labour and may be injured during fetal expulsion. It should not be difficult to identify the everted bladder. It is pear-shaped and attached to the vaginal floor; urine drips from the two openings of the ureters, and the congested mucosa is apparent. Epidural anaesthesia should be induced. The bladder is first cleaned, and any lacerations are repaired by suture. The organ is then compressed between both hands and gradually forced back into the urethra. Further manipulation is then applied to the vaginal floor until the bladder is properly replaced. Antibiotic therapy lasting several days should be prescribed. Tetanus antitoxin should be given. Prolapse of the bladder occasionally occurs in cattle, but is rare.

Protrusion of the Bladder

Protrusion of the bladder through a rupture in the wall of the vagina may occur during or after parturition (see also Chapter 13, Fig. 13.2). The rounded organ protrudes from the vulva. The kink that forms in the urethra prevents micturition; thus the organ progressively distends with urine. The condition must be distinguished from prolapse of the vagina, cyst or tumour of the vagina, haematoma of the vulva, and prolapse of perivaginal fat. The surface of the bladder is cleaned, and the organ is punctured with a hypodermic needle to allow drainage of urine. It is then dressed with an antibiotic powder and gently pushed back into place through the vaginal rupture. The latter is then repaired. Epidural anaesthesia will greatly facilitate return of the protruded organ.

Prolapse of Perivaginal Fat

Prolapse of perivaginal fat is most likely in over-fat heifers of beef or dairy breeds and is a sequel to a rupture of the vagina, often a small one. The site of rupture is typically at the junction between the vagina and vestibule (Arbeiter & Mayer 1967). The fat should be carefully removed with scissors or an emasculator, and if possible, the vaginal tear should be sutured. Recently, Blaser et al. (2013) described an alternative method in which deep sutures are placed into the pedicle of the protruded fat, after which the fat is removed with an ecraseur. The defect in the vaginal wall is then closed with the previously placed sutures.

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• **Fig. 20.11** Infarction of the colon is a rare complication of prolapse of the rectum in the parturient mare.

Prolapse of the Rectum

Slight eversion of the rectum is a common accompaniment of powerful expulsive efforts, which usually recedes after delivery. Severe prolapse is likely only in the mare; if it is already present in a dystocia case when the veterinary surgeon arrives, an attempt should be made to reduce the prolapse, and an assistant should be instructed to maintain the organ in position by pressing a towel against the mare's anus. Epidural anaesthesia may be needed to replace the rectum. When the prolapse has been present for some hours before veterinary assistance is available and the organ has become markedly oedematous and contused or torn, it may be difficult or impossible to replace. Submucous resection under epidural anaesthesia or under a general anaesthetic must then be carried out. In the mare, parturient prolapse of the rectum, no matter how transient, may prove fatal because stretching or tearing of the colic mesentery can result in infarction of the terminal colon (Fig. 20.11). The affected segment of bowel becomes atonic, defecation stops, and the mare's condition deteriorates insidiously during the next few days.

Postparturient Prolapse of the Uterus

Prolapse of the uterus is a common complication of the third stage of labour in the cow and, less commonly, of the ewe. It occurs less frequently in the sow and is rare in the mare and bitch. In the ruminant species the prolapse is generally a complete eversion of the gravid horn, whereas in the sow and the bitch eversion is generally partial and comprises one horn only. Cases are on record in which the bitch has everted one horn before she has completely delivered the fetuses from the other. In the mare, the rare cases of prolapse are generally partial only.

• **Fig. 20.12** Uterine prolapse in a cow in lateral recumbency. The fetal membranes are still attached and there is a significant degree of rumen tympany. (Courtesy Dr. Jakob Malmo.)

Cow

The prevalence has been reported as ranging from 0.2% in range beef cattle in America (Patterson et al. 1979) to 0.3% in Scandinavian dairy cattle (Odegaard 1977, Roine & Soloniemi 1978) to 0.09% in dairy cows in California (Gardner et al. 1990). The occurrence seems to be affected by seasonal as well as regional factors, the condition being more common in the autumn and winter (Gardner et al. 1990) in some years and in some localities. Pluriparous animals of the dairy breeds are more prone to suffer from the disorder than are heifers, although in a study by Murphy and Dobson (2002) in the UK, the disorder was more prevalent in beef breed heifers than in cows. The highest incidence in dairy cows in New Zealand was in fourth parity animals (Oakley 1992).

In the majority of instances the prolapse occurs within a few hours (over 90% in less than 24 hours after calving; Gardner et al. 1990) of an otherwise normal second stage labour, although Gardner et al. (1990) reported that in their study, 47 out of 200 cases had received obstetrical assistance; similarly, Murphy and Dobson (2002) found that 33% of their cases followed an assisted calving. In some cases prolapse may be delayed for several days; these are generally associated with a grossly protracted and assisted labour. When delivery is achieved by heavy traction, the uterus sometimes prolapses immediately after the calf is withdrawn. Likewise, some cattle with extreme laxity of the perineum and vulva may spontaneously prolapse immediately after every calving.

The signs of this condition are obvious. As a rule, the affected cow is recumbent and if in lateral recumbency, rumen tympany can be prominent (Fig. 20.12); less often, the cow is standing with the everted organ hanging down almost to its hocks (Fig. 20.13).

Aetiology

Prolapse of the uterus occurs during the third stage of labour, normally within a few hours of the expulsion of the calf, and at a time when some of the fetal cotyledons have separated from the maternal caruncles. The aetiology of the condition is not fully elucidated but is clearly multifactorial (Dawson & Peter 2012).

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• **Fig. 20.13** Uterine prolapse in a standing cow. Note that the fetal membranes have been shed. (Courtesy Dr. Jakob Malmo.)

• **Fig. 20.14** Uterine prolapse in which there has been haemorrhage from a torn uterine blood vessel into the everted organ. The prognosis for such cases is extremely poor. (Courtesy Dr. Jakob Malmo.)

Many of these factors relate to uterine inertia. Thus causes of primary inertia, such as hypocalcaemia, impairment of expression of prostaglandin or oxytocin receptors, and myometrial defects due to overstretching, contribute to primary uterine inertia, whereas exhaustion and dystocia contribute to secondary inertia. All of these contribute to the risk of uterine prolapse. Hypocalcaemia may also make the uterus atonic after the end of labour, resulting in delays to the involution of the cervix and uterus. Secondly, when tenesmus continues after second stage labour, there is increased risk of prolapse. Such situations include the aftermath of a dystocia, forced extraction of the fetus to resolve fetomaternal disproportion, retained fetal membranes, and laceration of the reproduction tract. Thirdly, prolonged recumbency, either due to paralysis or hypocalcaemia, is associated with the risk of prolapse, possibly due to the rise in intraabdominal pressure that is consequent upon being recumbent. High intra-abdominal pressure may also underlie the increased risk of prolapse in overconditioned or obese animals. Outbreaks of uterine prolapse have also been attributed to extreme weather, deficiencies of phosphorous or magnesium, or the presence of oestrogens in pasture. Finally, prolapse is more common in closely confined and highly fed cows rather than those at range.

Regardless of the predisposing factors, it appears that the onset of uterine inversion and prolapse occurs during or after third stage labour when a portion of detached afterbirth occupies the birth canal and protrudes from the vulva. Vandeplassche and Spincemaille (1963) were of the opinion that the pregnant horn does not undergo a progressive inversion from its anterior extremity; only the posterior two-thirds invert. The actual protrusion of this portion can occur very quickly in one bout of straining.

Prognosis

The prognosis will depend on the type of case, the duration of the condition before it is treated, whether the organ has sustained severe injury, whether haemorrhage has occurred from a major uterine blood vessel, and whether other organs (bladder, intestine) are involved in the prolapse. The mortality rate for a cow with an uncomplicated prolapse has been reported as 18% to 20% (Odegaard 1977, Gardner et al. 1990, Oakley 1992) within 24 hours of treatment; in addition, Oakley (1992) also found that a further 15% of animals died or were lost from the study during the course of the calving season. The prognosis for animals in which the prolapse has been long standing, in which there is intestinal or bladder involvement in the prolapse, or in which haemorrhage has occurred, is generally poor. Immediate recurrence occurs in a small proportion of animals: failure to completely extend the tips of the uterine horns is particularly associated with an increased risk of reprolapse.

Fertility of cows that survive uterine prolapse is reduced compared with normal cows. Patterson et al. (1979) reported that 40% of cows became pregnant after uterine prolapse. Murphy and Dobson (2002) found that only 55% of cows that had suffered from uterine prolapse were bred again, compared with 91.7% for unaffected cows; but, of the animals that were rebred, conception rates were satisfactory at 75% (versus 80.7%). Intercalving interval was extended in the studies of Jubb et al. (1990) and Oakley (1992) by 10 and 50 days, respectively. Loss of pregnancies in midgestation was also reported in the latter studies. Most cows that suffer a uterine prolapse calve subsequently without trouble. Murphy and Dobson (2002) reported only one recurrence at the next calving out of 77 cows that survived a prolapse, whereas Jubb et al. (1990) reported finding one cow that had prolapsed twice.

Haemorrhage is a serious potential complication of prolapse. Haemorrhage can occur when the uterine is prolapsed (Fig. 20.14) or, occasionally, after its replacement. Postmortem examination of such cases indicates that haemorrhage occurred due to the weight of the everted organ having torn the mesovarium and the ovarian artery.

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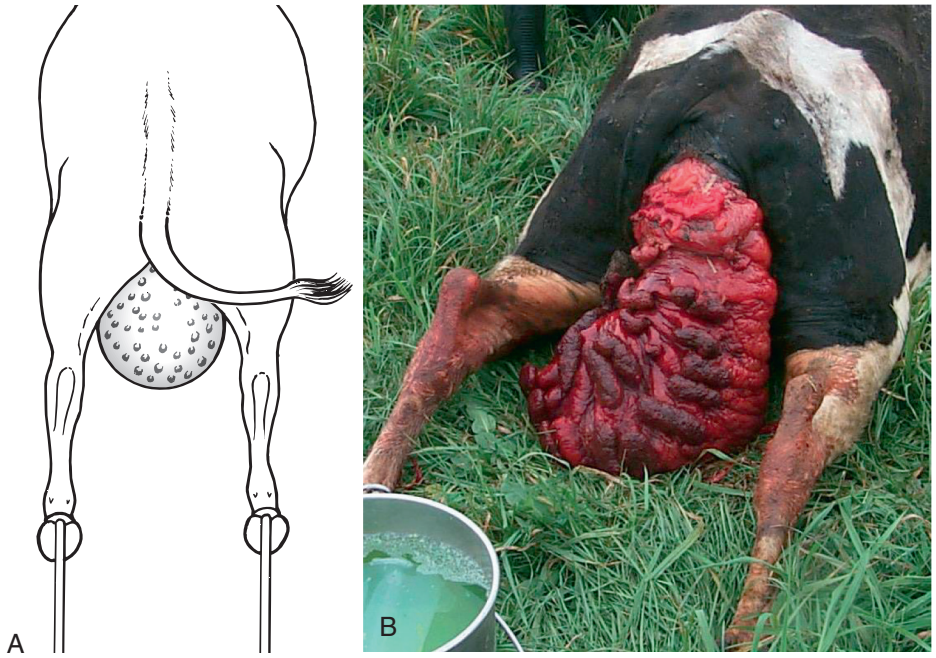
Treatment

The main difficulties in replacement of the organ are: (i) the almost continuous straining which manipulation of the uterus provokes; (ii) the pressure that has to be applied in an uphill direction; and (iii) the oedema/congestion of the uterus due to compression of the venous return.

Straining is prevented by the administration of an epidural anaesthetic. This also has the advantage that defecation is in abeyance during the operation. It is good practice to give a preliminary injection of calcium borogluconate (as for milk fever) and to relieve rumen tympany.

The need for uphill pressure can be managed by raising the hindquarters of a recumbent animal (e.g., by placing on a bale of straw or by using hip lifters), although the method described by Plenderleith (1986) is now almost universally used for recumbent cows. The cow is placed in sternal recumbency with both hindlegs pulled out behind her (weight therefore being taken on her stifles) (Fig. 20.15A and B; 'froglike position'). The assistant sits astride the cow, facing the rear, and holds the cow's tail up vertically. This manoeuvre causes the slope of the vulva to be upwards. The veterinarian then kneels between the cow's hocks and supports the weight of the prolapsed organ on his or her thighs. The weight of the uterus can be further supported by an assistant holding the corners of a towel, a plastic sack, or a piece of board. If the cow is standing, it is very helpful indeed to have to assistants holding the uterus above the level of the vulva by such means. If the fetal membranes are still attached, it is worth making an effort to remove them to reduce the weight of the uterus and reduce the risk of reprolapse. If the fetal membranes are already partially detached and their complete removal can be carried out easily and without injury to the caruncles, this should be done. However, if attachment is complete or when attempts at detachment are associated with haemorrhage, it is better that the organ be replaced with the membranes still adherent. (The subsequent management of the retained fetal membranes should be on the principles outlined in Chapter 23). The prolapsed organ should be palpated in order to detect the possible presence within it of a distended urinary bladder; if such is the case, the bladder should be emptied by the use of a catheter.

Wapenaar et al. (2011) obtained a consensus view of the management of uterine prolapse from a Delphi survey of expert opinion as follows. Before arrival, the uterus should be protected and supported by wrapping it in a moist towel or sheet, and the animal should be restrained where it is. Epidural anaesthesia should be administered plus a sedative if the animal is fractious. Assistance to restrain and support the cow and support the uterus should be sought. If possible, the cow should be standing, but if she is recumbent, she should be placed in the froglike position previously described. Lateral recumbency should be avoided; cows that are standing should be left standing, but those that are recumbent should be left recumbent. Recumbent cows that rise are at risk of rupturing uterine blood vessels as they move around, especially if they are uncoordinated due to early stage milk fever (Dawson & Peter 2012). In this context, it is worth noting that the prognosis for standing cows appears to be better than for recumbent animals: Ishii et al. (2010) reported a survival rate of 65% for standing cows versus 38% for recumbent animals. The uterus and perineum should be cleaned with warm, clean water, and the tail tied to one side. The uterus should be checked for lacerations: partial thickness lacerations should be sutured if they present a risk of haemorrhage or perforation but not otherwise. Full thickness lacerations should be sutured, but, if they are extensive and the cow's condition is poor, culling may be a better alternative. Lubrication is useful if the uterus is dry or oedematous, but the use of hygroscopic substances such as sugar or salt to draw out



• **Fig. 20.15** Positioning a recumbent cow for replacement of a prolapsed uterus. The cow is positioned in sternal recumbency, with the hindlegs extended backwards and the prolapsed organ situated between them. (A) Diagrammatic representation based on Plenderleith (1986). (B) Field case. ((B) Courtesy Dr. Jakob Malmo.)

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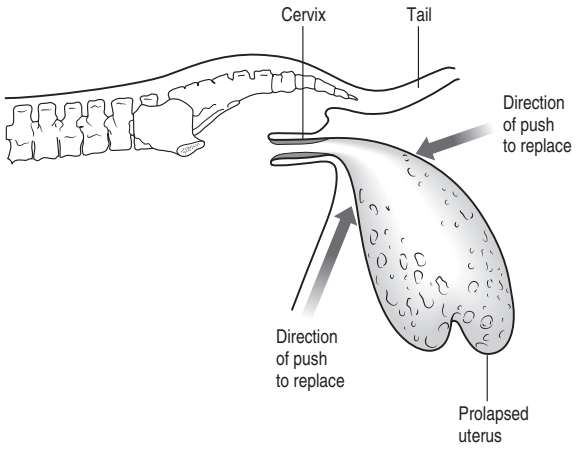

• **Fig. 20.16** Diagram illustrating where pressure is applied to start the replacement of the prolapsed uterus.

oedema is not recommended. The fetal membranes should be removed according to the criteria described previously. Replacement should normally start at the vulva.

During replacement of the uterus, the veterinarian starts to replace the uterus little by little, starting with those portions that are nearest the vulval lips (Fig. 20.16). The nearest cotyledons are pushed into the vagina, taking care that the lips of the vulva remain well apart and do not become turned inwards. It is generally best to replace portions of the upper and lower surfaces alternately. When the last portions remain to be replaced, an assistant should press against these, using the palms of both hands, while the veterinarian endeavours to draw the lips of the vulva over the prolapse. As the mass disappears through the lips of the vulva, the veterinarian, using a clenched fist, should then continue to press it forward to the full length of the arm, ensuring that the uterus is pressed forwards beyond the cervical ring and to ensure that the horns are fully inverted. A bottle or blunt probang may be helpful with this step. It is worth taking time to ensure that the horns are fully inverted, as failure to invert the horns is significantly associated with the risk of re-prolapse. When this has been accomplished, the cervix should lie unoccupied at the level of the pelvic brim and, if the whole uterus has passed the cervix, it should regain its normal position. To ensure complete replacement of the uterus, it can be helpful if 9 to 14 litres of clean warm water are infused into the uterus by gravity feed and immediately removed by siphonage; the weight of water eliminating any remaining inversion of the horn.

After replacement, oxytocin should be given to help restore uterine tone and thus to prevent recurrence of the prolapse. It is generally held that preoperative treatment with oxytocin, although reducing the size of the prolapsed organ, increases the turgidity of the everted organ and makes replacement more difficult. However, a method of direct injection of oxytocin into the uterine muscle before attempting replacement has been described, which is said to make the process of replacement easier. Even if the animal shows no clinical signs of hypocalcaemia, IV calcium borogluconate therapy should be given, together with parenteral antibiotics and a nonsteroidal antiinflammatory agent. Intrauterine antibiotics are not generally found to be helpful.

A final advantage of caudal epidural anaesthesia is that straining will be prevented for an hour or so after replacement of the organ; the duration will be extended if xylazine is used as well. Some veterinarians choose to insert vulva sutures in a Buhner pattern to reduce the risk of reprolapse. This practice is controversial; many consider that it serves no useful purpose because prolapse should not recur if it has been replaced correctly. It may even stimulate the cow to strain, allowing the prolapse to recur within the animal and thus not be detected. Others consider that, provided the cow is reexamined 24 hours later and the sutures are removed, it can prevent the recurrence of a complete prolapse that will be much more difficult to replace a second time. In uncomplicated cases it is generally found that within 24 hours of replacement the degree of cervical contraction present is such that recurrence is very unlikely. Wapenaar et al. (2011) suggested that such sutures should be removed 4 to 5 days after replacement.

Amputation of the Uterus

Uterine amputation is a procedure for the salvage of the cow in which the uterus is necrotic or severely traumatised. The surgery can be performed using an open or closed approach. An open approach allows the identification and replacement of any viscera that are within the prolapse before the uterus is amputated, whereas the closed approach is rather simpler (Prado et al. 2016).

Dawson and Peter (2012) describe the open surgical procedure as follows. The uterine body is incised from the cranial cervix to the bifurcation of the uterine horns to expose the contents of the prolapse. Any abdominal viscera are returned to the abdominal cavity. The major uterine vessels are then located in the stretched broad ligament and are double ligated. The vessels are severed between the ligatures, and the mesometrium is incised and allowed to retract into the abdominal cavity. Next, a series of adjacent, slightly overlapping mattress sutures are placed across the uterine body just proximal to the cervix, after which the uterus is amputated 2 to 3 cm below this line of sutures. The stump is then sutured with a simple continuous pattern.

In the closed approach the entire organ is circumferentially tightly ligated near the vulva with surgical tubing, broad suture (umbilical tape), or elastic/rubber tubing. The uterus can either be allowed to slough over a period of 7 to 10 days or can be amputated straight away and the stump returned to the abdomen. If the uterus is allowed to slough, there is a risk of exsanguination due to the weight of the prolapse (Meisner 2009).

Postoperative treatment should include administration of tetanus toxoid, nonsteroidal antiinflammatory drugs, and appropriate antibiotics.

Sheep and Goat

The method of replacement is similar to that described for the cow (Figs 20.17–20.20), except that it is easier to perform because of the ease with which the hindquarters of the ewe or doe can be kept raised by an assistant; caudal epidural anaesthesia should always be used except in those situations in which a long delay may occur before it could be treated by a veterinarian. However, because of their different physical relationship to the caruncles, the fetal cotyledons cannot readily be detached, and rather than damage the uterus by persistent attempts to separate them, it is preferable to leave them attached and return them with the uterus; failure to detach them at this stage will not significantly affect the prognosis. Anaerobic infection should be anticipated and prophylactic antibiotic used. Murphy and Dobson (2002) noted that, among sheep farmers, there is a reluctance to retain a ewe for breeding that has suffered from prolapse for fear of it recurring.

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• **Fig. 20.17** Uterine prolapse in a doe goat; fetal membranes have been shed. (Courtesy Dr. Yoav Alony.)

• **Fig. 20.18** Uterine prolapse in a doe goat; under caudal epidural anaesthesia the uterus is carefully and gradually being replaced. (Courtesy Dr. Yoav Alony.)

• **Fig. 20.19** The prolapsed uterus of the doe goat has been completely replaced. (Courtesy Dr. Yoav Alony.)

• **Fig. 20.20** A perivulval purse-string suture is being inserted to ensure retention of the uterus. (Courtesy Dr. Yoav Alony.)

Horse

Aetiology

The disorder is relatively uncommon in this species, but when it occurs, it can be life-threatening (Pascoe & Pascoe 1988). The condition is more common in older, pluriparous mares and in mares that have experienced prolonged dystocia and/or delivery of the fetus by traction (e.g., Hadiya et al. 2015). The condition is also related to partial or complete failure of expulsion of the fetal membranes (MacGillivray 2011). The membranes tend to separate from the endometrium in the uterine body more readily than at the tip of the uterine horns. The consequence of this is that, during the persistence of uterine contractions during third stage labour, the pull on the attached membranes at these points cause the eversion of the tip or tips of the horns. The continued uterine contractions and the subsequent straining as the mass of the fetal membranes enter the pelvis cause the whole of the uterus to be inverted and prolapsed. Mares that have to undergo Caesarean hysterotomy may also be at risk of uterine prolapse if the fetal membranes are accidentally sutured into the hysterotomy wound or if they remain attached at the tip of the uterine horn (Arthur & Allen 1971, unpublished). Hence it is important that, when closing the uterus, the allantochorion should be separated from the endometrium for some distance (see Chapter 16) around the site of incision. Some cases of uterine prolapse occur if retained fetal membranes are delivered by traction; eversion of the uterus at the point of attachment of the fetal membranes can be quickly converted into a prolapse when the mare strains. Thus in spontaneously occurring cases of uterine prolapse, the weight of the fetal membranes hanging from the vulva and the straining that occurs as a consequence of failure of passage of the fetal membranes are important causative factors in the development of uterine prolapse. In view of these observations, it is important that undue traction on the detached portion of the fetal membranes should not be applied when the more anterior retained portion is being freed. It is for this reason that the use of an oxytocin drip is the preferred method of treating retained fetal membranes in the mare (see Chapter 16).

Treatment

Diagnosis of a complete prolapse is straightforward. However, some mares have a partial prolapse of intussusception of a uterine horn, which is more difficulty to identify; these cases may present with persistent tenesmus, restlessness, colic, and tachycardia (Turner 2007) rather than overt uterine prolapse. The presence of lacerations, ischaemia, or necrosis of the uterus is associated with a poor prognosis for survival.

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The approach to replacement of the prolapse is broadly similar to that described previously for the cow. As soon as the condition is identified, the prolapsed tissue should be elevated/supported by two assistants to prevent the development of passive venous congestion and oedema. The mare must be adequately restrained to prevent trauma to the prolapsed organ and to prevent injury to the veterinarian. Sedation, IV analgesia, and caudal epidural anaesthesia (Chapter 14) are required in almost all cases; general anaesthesia may be required if the mare is fractious. Before replacement, an attempt should be made to remove the fetal membrane so long as the allantochorion can be readily separated from the endometrium without causing haemorrhage; if this is excessive, then as much as possible should be cut away before replacement. Also, the prolapse should be palpated to ensure that the bladder is not entrapped within it: if this has occurred, the bladder will need to be emptied via a catheter before the uterus is replaced.

The uterus should be replaced starting at the part adjacent to the vulva as described in the cow. The technique is easier than in the cow because of the absence of the caruncles; this tends to reduce the amount of haemorrhage. After replacement it is important to ensure that the organ is completely inverted; an intrauterine infusion of saline, with subsequent removal by siphoning, can be used, or a mare vaginal speculum can be inserted into the length of the uterine horn (Turner 2007). Once the organ has been replaced, the mare should be given oxytocin to restore tone to the uterus. Systemic antibiotics as well as antiinflammatory agents should be used, and there is a relatively high probability of laminitis occurring. Vulval sutures should never be used.

Pig

The consensus of veterinary opinion is that pigs are unable to tolerate uterine prolapse unless the uterus is replaced easily and quickly; frequently, by the time help is summoned, the sow will have died most probably due to a fatal haemorrhage after rupture of the uterine vessels or possibly shock. The degree of prolapse will vary from part of one horn at its simplest to both horns at its most extreme. The sow should be deeply sedated or preferably given a general anaesthetic and placed in an incline with the head facing downwards or suspended by her hindlegs. If the uterus is traumatised, then euthanasia is preferable, particularly in commercial pig units because there will be a delay before she can be served by the boar and become pregnant. An alternative procedure which merits a trial is to 'float' the uterus back into the abdomen with the aid of water pressure. The sow is placed on her side, head downwards, on a slope; the end of a soft tube of rubber or plastic, 2 cm diameter and 1.5 m long, is gently passed into the stoma of the prolapsed viscus and eased along as far as possible. Clean water is then allowed to flow into the prolapsed viscus. The weight of the introduced water gradually draws the prolapsed organ back into the abdomen; the tube is introduced further and more fluid infused. By this means the whole uterus is not only returned but completely replaced without manipulation. In the case of noncommercial pet sows, replacement can be attempted under general anaesthesia via a laparotomy (Raleigh 1977), as described later in this chapter for the bitch.

Dog and Cat

A laparotomy can be performed, and with simultaneous external manipulation and abdominal taxis, replacement can be attempted. It is more usual, however, to carry out 'external' hysterectomy on the prolapsed organ. The prognosis is favourable after amputation.

Parturient Recumbency

Recumbency, as a complication of parturition, is occasionally seen in all species but is most common and important in cattle.

Prepartum Recumbency in Cattle

The main causes of prepartum recumbency in cattle are nutritional: starvation ketosis (protein–energy malnutrition) and pregnancy toxaemia.

Starvation ketosis is used to describe inadequate supply of feed in terms of quantity, quality, or both. In such conditions of general underfeeding, the resulting protein and energy deficiencies cannot be strictly separated. The condition is commonly seen in heavily pregnant cattle on a poor plane of nutrition, particularly towards the end of the winter when fodder is scarce or poorly saved. Beef cattle on hill farms are at the highest risk of developing the condition. Affected animals are usually bright and alert and have normal appetites, and cardinal signs are generally normal; however, emaciation can be extreme. The onset of energy deficiency is gradual; thus neither ketone nor NEFA concentrations are particularly elevated (Malmo et al. 2010).

Animals that are recumbent generally have a poor prognosis and, in repunctuate animals that are in lateral recumbency, the prognosis is virtually hopeless. If treatment is attempted, it is based on providing additional feed, supplemented with propylene glycol, and attempts to lift the animal from recumbency. Premature induction of calving with corticosteroids can be attempted if the animal's condition is not too severe, but the stress of the induced calving can be fatal to more severely affected animals. Otherwise, in the interest of the animal's welfare, euthanasia should be performed, with salvage recovery of the calf (if it is likely to be viable) and measures taken to ensure that similar cases do not recur.

Pregnancy Toxaemia of Beef Cattle

Pregnancy toxaemia is most often seen in fat or very fat pregnant beef cows which are grazing marginal land or are provided with a much reduced plane of nutrition during the last 1 or 2 months of pregnancy. It can occur in animals carrying a single large calf or carrying twins. Growing heifers are particularly at risk due to the competing demands of pregnancy and growth. The resultant large scale mobilisation of body fat results in severe ketonaemia and fatty infiltration of the liver. Affected animals show signs of severe ketosis, including dullness, depression, inappetence, rapid weight loss, and, sometimes, recumbency. Blood concentrations of β–hydroxy butyrate and liver enzymes are typically high, and animals are hypoglycaemic. The response of severely affected animals to treatment is often poor, but less severely affected animals may respond to standard therapies for ketosis. Immediate removal of the calf by Caesarean hysterotomy may save a valuable cow (Malmo et al. 2010).

Postpartum Recumbency

Hypocalcaemia

Hypocalcaemia is the chief cause of recumbency in parturient and puerperal cows. It is a metabolic disease that is characterised

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by the development of paresis and severe hypocalcaemia near the time of calving. Clinically, it is characterised by weakness, recumbency, depression of consciousness, and, ultimately, death. Approximately 75% of cases occur within 24 hours of calving. Most cases occur within 48 hours of calving, but cases can occur several days before calving and up to 10 days after calving (Malmo et al. 2010). Parturient hypocalcaemia needs to be differentiated from other puerperal conditions, including acute coliform mastitis, calving paralysis, acute hypomagnesaemia, uterine disorders such as puerperal metritis, uterine rupture or retention of the fetus, or generalised peritonitis. Reference should be made to standard textbooks of bovine medicine for a full description of the condition.

Puerperal Metritis and Toxaemia

Puerperal metritis usually follows dystocia and is often accompanied by retention of the afterbirth. There is a fetid vulval discharge and diarrhoea; straining is frequent, and there is an expiratory grunt. The pulse rate is elevated, but the temperature, although at first raised, may be falling in a case of advanced toxaemia. A physical examination should verify the suspicion of metritis as a cause of recumbency. The clinical signs and treatment of puerperal metritis are described and discussed in Chapter 23.

Puerperal Laminitis

Puerperal laminitis is a troublesome complication of puerperal metritis. It is essentially an equine condition, but the other farm animals are occasionally affected. In the mare the condition is a likely sequel to retention of the fetal membranes. Two to 4 days after foaling the typical stance of laminitis is seen, the hindlegs being placed well forward to ease the weight on the more severely affected forefeet. It is a most painful condition and causes rapid loss of weight. Owing to the prolonged periods of recumbency and diminution in milk secretion, the foal may require artificial feeding. Avoidance of puerperal laminitis lies in preventing metritis by treating cases of dystocia promptly and carefully and by the appropriate treatment of retained fetal membranes.

Hypocalcaemia of Sows

True hypocalcaemia occurs occasionally in sows, but the most likely cause of postparturient recumbency is toxaemia due to metritis or mastitis. Incomplete parturition with retention of a fetus or a portion of the afterbirth should always be suspected. Failure of milk secretion is one of the symptoms of toxaemia and hypocalcaemia; it sometimes results from lack of the 'let-down stimulus'. So-called agalactia of sows is thus not a specific syndrome but merely a symptom common to several quite different affections.

Physical Inability to Rise

Physical inability to rise may be due to muscular weakness or to lesions of the locomotor system. Locomotor lesions that may occur during labour and cause recumbency include: dislocations of the hip and of the sacroiliac joints; fracture of the pelvis, femur, or vertebral column; rupture of the gastrocnemius muscle; and paralysis of the obturator or gluteal nerves. When a large fetus is forcibly drawn into the maternal pelvis, the lumbar nerves, which pass over the lumbosacral joint to form the anterior part of the lumbosacral plexus, may be damaged; paralysis of the gluteal, sciatic, or obturator nerves is a possible result. This is particularly likely when an oversized fetus becomes impacted in a state of 'hiplock' (see Chapter 14), the nerves being trapped between the lumbosacral promontory of the mother and the iliac bones of the calf. In addition, the obturator nerve, as it passes down the inner surface of the iliac shaft, may be damaged by an oversized fetus.

A diagnosis of disease of the locomotor system depends on a methodical clinical examination with a view to eliminating the several possibilities. The degree and form of the disability and the manner of the unsuccessful attempt to rise often give a strong indication of the cause. The examination includes the manipulation of the hindlimbs to determine the presence of excessive mobility or crepitus; it is combined with a rectal examination of the pelvic bones. Regional absence of peripheral sensation may verify nerve paralysis, including paraplegia associated with vertebral fracture. In cases of recumbency due to physical inability or pain associated with attempts to rise, the affected animal is usually bright, its appetite is good, and, when undisturbed, its temperature and pulse are unaffected. Each case must be treated on its merits.

When no cause of recumbency can be found in an animal that appears normal in other respects, tissue swelling, oedema, or haemorrhage in the vicinity of nerves is possible. If such were the case, the normal recovery processes would diminish pressure on the nerves, and this would be reflected in progressively better attempts to rise. However, if a cow is still unable to rise after being recumbent for a week, the prognosis is grave. Slings, hoists, and other devices are sometimes used to encourage the patient to stand, but in general they are of limited use. The best contribution that can be made to a recovery is the provision of first class nursing. This comprises placing the recumbent animal on ample, soft, clean bedding that overlies a dry floor and that is frequently changed. The patient is turned from side to side as often as possible, with concurrent massage of the limb muscles. Meanwhile, close veterinary attention is paid to the health of the cow's uterus and udder.

Gluteal Paralysis

Gluteal paralysis is seen in the mare and cow; in the mare it occurs after a spontaneous birth. It is recognised when the dam is found to have difficulty in rising and when she walks with 'weakness of the hindlimbs'. Later, atrophy of the gluteal muscles is apparent. Prognosis is favourable, the disability usually disappearing in a few weeks, although occasionally complete recovery may take months. In warm weather, the affected animal should be placed in a paddock which is free from ditches and obstacles; here a firmer foothold for getting up is more likely than in a barn or loose box. The animal may be helped to rise by lifting on its tail and then steadying its hindquarters. In order that the mare may suckle the foal and also rest on its feet, slings may be usefully employed. If the mare or cow cannot get up within a few days of parturition the prognosis is grave.

Damage to the Sciatic Nerve and Its Branches

In the cow, damage to the sciatic nerve is associated with intrapelvic pressure damage during calving that occurs when the sixth lumbar nerve is compressed against the ridge on the sacrum before it joins the first two sacral roots to form the sciatic nerve (Vermunt et al. 2010). Animals with damage to the entire sciatic nerve typically stand with the fetlock knuckled over and the hock dropped (Fig. 20.21). When paralysis is complete, the limb is dragged and advanced by flexion of the hip only; weight may be borne on the dorsal aspect of the fetlock. Femoral and peroneal paralysis is not usually direct sequelae of birth; damage to the femoral nerve can occur when a recumbent cow struggles to rise when its limbs are

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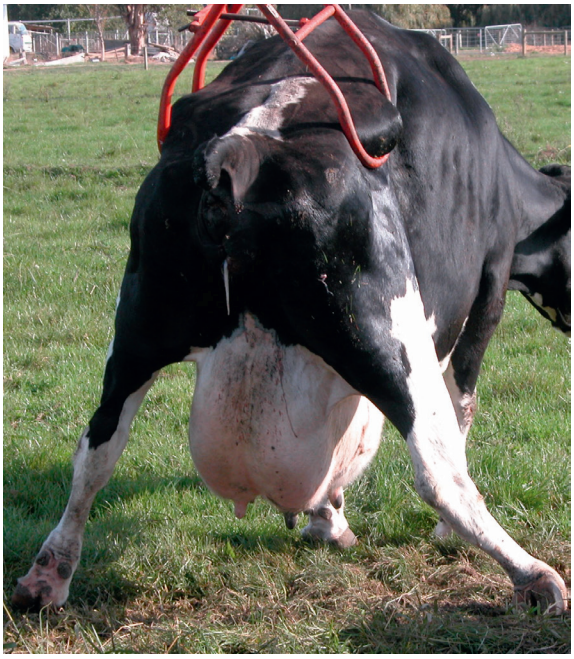
• **Fig. 20.21** Sciatic nerve paralysis associated with dystocia due to gross fetomaternal disproportion. (Courtesy Dr. Jakob Malmo.)



• **Fig. 20.22** Obturator paralysis is characterised by an inability to adduct the affected limb (in this case both hindlimbs are affected). (Courtesy Dr. Jakob Malmo.)

retracted caudally, whereas the peroneal nerve can be damaged by prolonged recumbency.

Obturator Paralysis

Obturator paralysis is more frequent in cows than mares. Obturator paralysis commonly occurs with the delivery of an oversized calf, causing compression of the nerve roots of L5 and L6 or compressing the obturator nerve as it courses down the medial shaft of the ilium (Vermunt et al. 2010). The obturator nerve supplies the adductor muscles of the thigh, thus paralysis results in abduction of the affected limb(s) (i.e., the animal is unable to bring the affected leg under the body, so it pushes out sideways; Fig. 20.22). When both nerves are damaged, the legs will be splayed, and the cow is unable to rise. If the cow is helped to its feet, the legs slide out laterally (in a characteristic 'froglike' position on its ventral abdomen with the hindlimbs flexed but abducted. When paralysis is one-sided, the cow generally requires assistance to rise, but, provided the affected leg is prevented from sliding outwards, it can usually remain standing. If the cow falls, there is a risk of limb fracture or dislocation of the hip joint. When there is complete and bilateral paralysis, prognosis should be guarded; when it is unilateral and the animal can walk with assistance, the outlook is favourable. If the limb is 'spread eagled', there is usually more severe damage than a simple obturator paralysis (Fig. 20.23). Such cases rarely recover (Vermunt et al. 2010). A significant proportion of cases show improvement within a few days and progress to a complete recovery, provided that they are provided with adequate nursing care and further damage to the limb is prevented. Hobbling together of the hindlegs with a strap applied above each fetlock prevents excessive abduction and secondary tearing of the adductor muscles or fracture of the femoral neck during attempts to stand. The cow must be assisted and maintained on her feet for milking

• **Fig. 20.23** Animals that are recumbent in a spread-eagled position have usually sustained more damage to the limb(s) than a simple obturator paralysis. (Courtesy Dr. Jakob Malmo.)

or suckling and as often as possible at other times. Necrosis of the muscles of the hindquarters must be prevented by the animal being turned from side to side, the hindquarters massaged, the bedding frequently changed, and the cow's rear and udder kept clean and dry. The frequent, careful, use of hip-lifters or a suitable sling can help maintain the circulation in the extremities and reduce the risk of muscle damage, although overuse can result in serious damage to the cow. Hip clamps should only be used if the animal can support its own weight on its hindlegs. Placing a rope under the brisket enables the cow to stand with greater comfort and for longer periods than if supported by clamps alone (Malmo et al. 2010). Slings are occasionally employed for cattle. Unless there is marked improvement within a fortnight, recovery is unlikely.

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21

Castration and Cryptorchid Surgery

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Castration of the Male Horse

Indications for Castration

Removal of the testes (also referred to as gelding, cutting, orchiectomy, orchidectomy) is routinely performed on male horses not intended for breeding purposes. The primary reason is to prevent stallion behaviour, which is generally regarded as inappropriate for most equestrian disciplines. In the wild, stallions organise into two distinct social groups: the harem bands that have one stallion and several mares, and the all male bachelor bands. Stallions can remain manageable if they remain in bachelor mode; however, interaction with other horses frequently results in harem band behaviour with increased aggression and reproductive drive (Klingel 1982). The average age that colts reach puberty is 69 weeks (range 55–101) (Cornwell et al. 1973). Stallions can be safely castrated at any age; however it is mostly undertaken at 6 months to 2 years of age. Some owners prefer to delay castration until after puberty to allow masculine features such as a crest or increased muscle mass to develop. In cattle, castration before puberty results in an increase in adult height due to delay in long bone growth plate closure (Shahin et al. 1992). It is possible that this also happens in the horse, but it is not documented or a reason for requesting castration by owners. Recently, there appears to have been an increasing tendency to castrate older animals once they have become unmanageable or once they have failed to perform to a level that justifies their use for breeding.

The anatomy of the reproductive organs, in particular the penis of the stallion, is shown in Fig. 21.1 and a diagram of the testes Fig. 21.2.

Surgical Techniques

Careful consideration of the age breed and type of horse, the premises at which the surgery is to be performed, and the methods for analgesia/anaesthesia should be made before selecting the surgical technique to be used. There are two broad surgical techniques, namely: (a) open castration (Fig. 21.3A–I) or (b) closed castration. An additional technique can be used in which an open castration is performed, and is subsequently closed; this prevents the possibility of postoperative herniation of abdominal viscera (Fig. 21.4 A–I). For an open castration, the vaginal tunics are incised, allowing direct visualisation of and access to the testicular vessels and the ductus deferens, which may then be ligated and crushed or solely crushed with an emasculator; the tunics are left open after removal of the testes (see Figs 21.3 A–I). For a closed castration, the vaginal tunics are not incised, with haemostasis ensured by placing ligatures around the spermatic cord (or transfixing ligatures); in this way no opening is created into the peritoneal cavity. For the open and subsequently closed technique (see Figs 21.4 A–I), the following procedure is performed: after opening the tunic, the blood vessels and ductus deferens are ligated and transaction, and the vaginal tunic and skin are subsequently closed.

Standing Castration

Standing castration (see Figs 21.3 A–I) can be undertaken in any colt that has normally descended testes, is large enough in height to enable the surgeon to access the scrotum by reaching underneath the abdomen, and has been well-handled with an amenable temperament. Owners should be warned about the risk of complications as they have been reported to occur in up to 60% of cases (Rosanowski et al. 2018).

The technique is traditionally used in thoroughbred racehorses in the UK but is less popular in sports horses and warm bloods. There is anecdotal evidence that warm bloods are more prone to scrotal hernia, and this may be the reason for this difference. Standing castration is generally performed using the open technique using emasculators and without placing ligatures across the vessels and ductus deferens. The major disadvantage of this technique is the possible risk of uncontrolled haemorrhage occurring and that it does not prevent the risk of evisceration of abdominal contents through the inguinal canal because the vaginal tunic is left open. However, the internal and external inguinal rings are not in alignment in the standing horse, and so the risk appears to be less compared with when the procedure is performed in the recumbent anaesthetised animal, when herniation may occur as the animal rises to a standing position.

The colt is first sedated, commonly using an α-2 adrenoceptor agonist (such as detomidine HCl) in combination with butorphanol tartrate; this results in profound sedation (Fig. 21.3A). The handler and surgeon remain on the left side of the colt, and the animal is made to stand against a wall on its right side. The surgeon approaches the scrotum from the horse's shoulder to reduce the chance of being kicked if the colt/stallion suddenly and unexpected kicks out with his left hind leg. The right (furthermost) testis is grasped in the left hand so that it is pushed down firmly into the scrotum. An 18-gauge 40 mm needle is advanced in one swift

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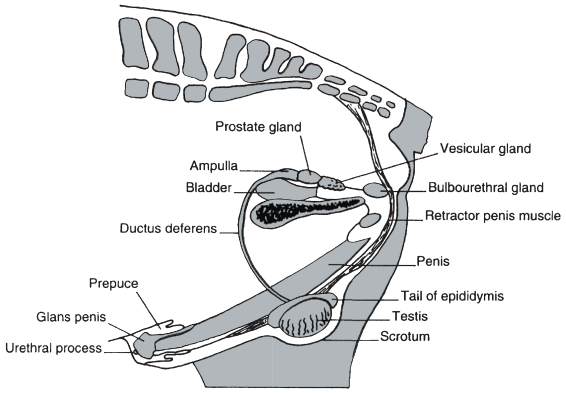
movement perpendicular to the skin of the scrotum directly into the testis to the level of the needle hub. Once the needle is in position, a 30 mL syringe containing the local anaesthetic agent is attached, and 20 mL of lignocaine HCl is injected into the testis. The needle is then retracted, and the remaining 10 ml is injected subcutaneously; then the needle is withdrawn (see Fig. 21.3B). The process is repeated for the left testis. Remarkably, this is well tolerated in the vast majority of animals. The scrotal skin can now be aseptically prepared. It is mandatory to wait after this; at least 10 minutes is allowed to elapse so that the local anaesthetic will have its maximal effect. Before commencing surgery the level of sedation should be assessed, and a further incremental dose of sedation may be required to ensure that the colt remains adequately restrained for the entire procedure.

The surgeon again grasps the left testis firmly against the scrotum, and a bold paramedian incision is made through the whole thickness of the scrotal wall (scrotal skin and vaginal parietal vaginal tunic) in a cranial to caudal direction, thus resulting in the 'open' castration technique. The incision is made large enough to allow the testis to be completely exposed. Gentle traction is then applied to the testis so that the 'opened' emasculators can be placed around the spermatic cord and testicular vessels with the cutting surface directly adjacent to the testis and the crushing surface positioned towards the direction of the spermatic vascular and not adjacent to the testis (the butterfly nut must be directed towards the testis (the so called 'nut to nut' orientation). The Serra emasculators are firmly closed at right angles to the spermatic cord, and the testis is removed. The emasculators must be closed completely, although this can require considerable force if the colt is large and the emasculators are not sharp. In these cases it may be best to first sever the caudal ligament of the epididymis with a scalpel, which allows the spermatic vessels and ductus deferens to be separated allowing the emasculators to be placed across the vessels and ductus separately (see Fig. 21.3 D). Once closed, the emasculators should be left in place for at least 5 minutes. During this time the procedure for removal of the left testis can commence. After removal of both testes, the scrotal incision is first inspected and if necessary manipulated to cover any protruding tissue; occasionally protruding connective tissue requires excision.

Routine perioperative antibiotics are administered systemically, along with non-steroidal antiinflammatory drugs. Procaine penicillin should not be used in horses likely to compete within 6 weeks; this mainly applies to race horses. Fly control is mandatory in the summer months to repel the attention from flies and prevent myiasis and other forms of wound contamination; many practitioners prefer to perform routine castration in the spring and autumn.

It is normal to advise that exercise is initially restricted for the first 36 hours postoperatively to only 'walking in hand', followed by a progressive return to exercise. Some trainers like to allow horses to swim shortly after castration, but although it may control swelling, this cannot be recommended due to the risk of wound contamination and even peritonitis.

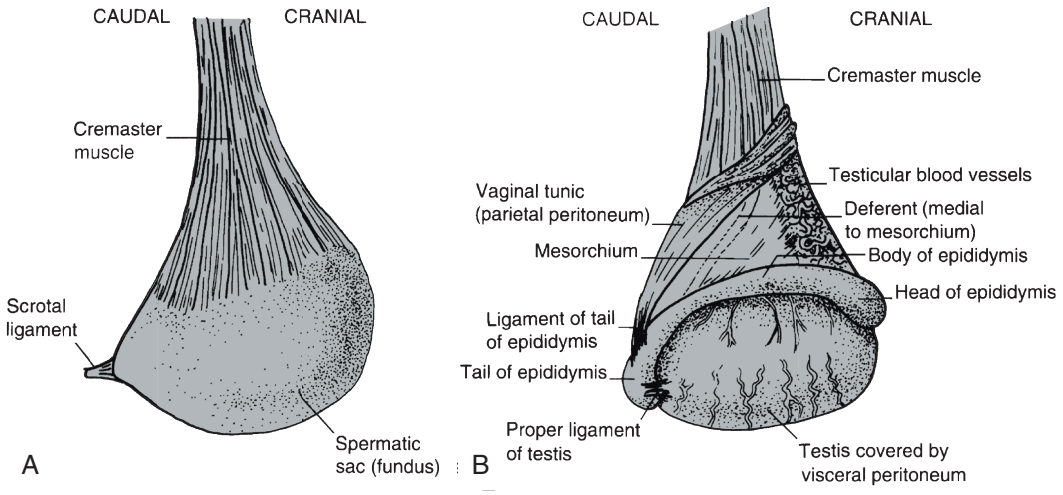


• **Fig. 21.1** Diagram of the anatomy of the reproductive organs of the stallion. (Redrawn with permission from Laing et al. 1988.)

Field Castration

Closed castration can be undertaken in the field or in a cleaner environment (e.g., a hospital induction box), or the open and subsequently closed method can be used. These methods are usually performed under dissociative anaesthesia and are appropriate for colts of 6 months to 2 years irrespective of temperament. It may also be used in mature stallions in which there are financial constraints when an increased risk of herniation has been identified.

The technique described here is that which is used routinely in the authors' hospital because this appears to address the most significant complications and has been associated with very low complication rates.



• **Fig. 21.2** Ligaments of the scrotum. (A) Lateral view of the right spermatic sac of the horse. (B) Lateral aspect of the contents of the right spermatic sac of the horse. (Redrawn with permission from Cox 1982.)

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• **Fig. 21.3** (A) Colt under sedation before standing castration. (B) The colt is adequately restrained, under sedation and standing, and local anaesthetic is injected into the scrotum. (C) The scrotal skin has been incised over the right testis. (D) The vaginal sac has been incised, and the surgeon has exteriorised the right testis and epididymis by gentle traction on both simultaneously. (E) The Serra emasculator has been applied to the spermatic cord of the right testis; note that the wing nut of the emasculator is adjacent to the testis. (F) The left testis and epididymis have been exteriorised by applying traction.

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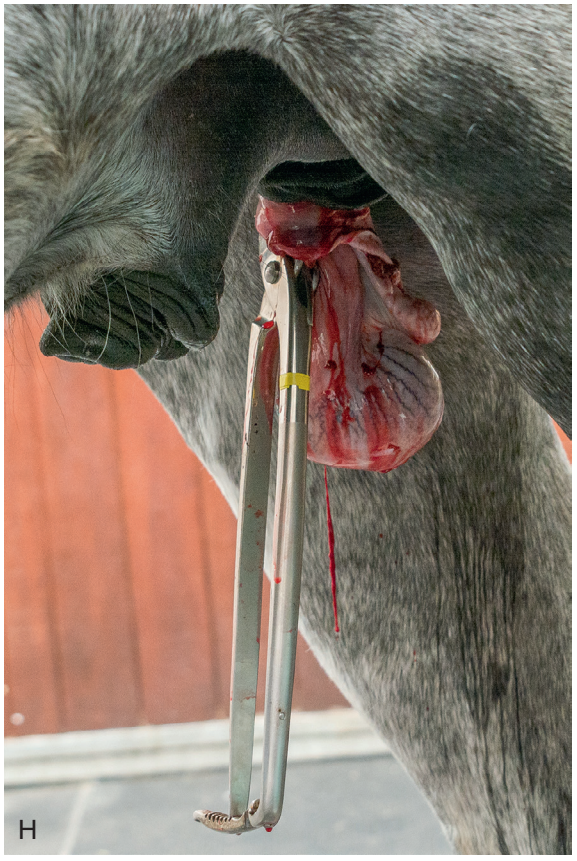
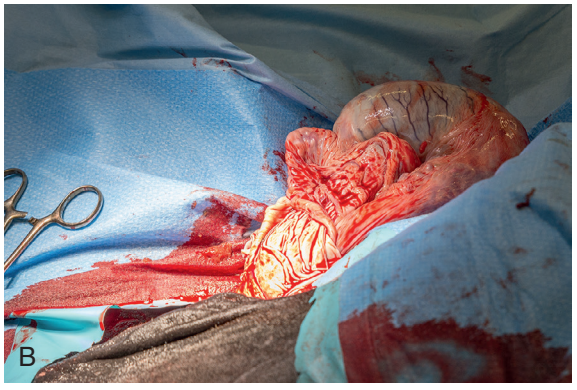


• **Fig. 21.3, cont'd** (G) The Serra emasculator has been applied to the left spermatic cord. (H) The emasculator is closed without severing the spermatic cord and remains attached before the jaws are finally completely closed to severe the cord. (I) The scrotal incisions after completion of castration.



• **Fig. 21.4** (A) The scrotal skin over the left testis has been incised, and with gentle pressure, the vaginal sac exposed. (B) The left testis is exteriorised.

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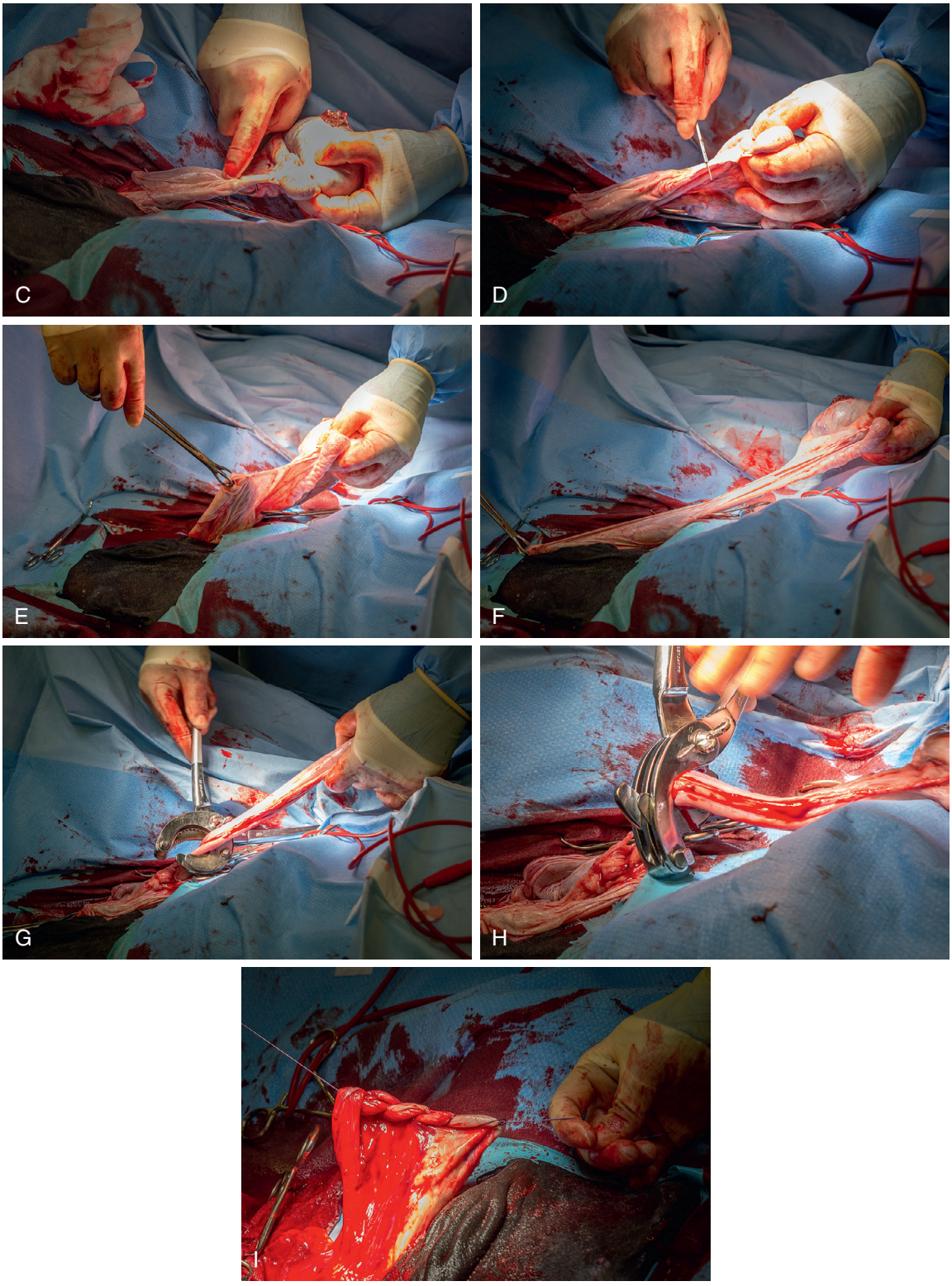

• **Fig. 21.4, cont'd** (C) The surgeon's index finger is pointing to the epididymal ligament. (D) The epididymal ligament is being incised with a scalpel. (E) The epididymal ligament has been incised, and the testis and epididymis withdrawn from the vaginal sac. (F) The testis and epididymis are further exteriorised. (G) The Serra emasculator is applied to the spermatic cord (note that the wing nut is directed towards the testis). (H) The emasculator had been closed to severe the spermatic cord. (I) After excision of the testis and epididymis, the vaginal sac is closed by a continuous suture.

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Although premedication with acepromazine is slightly controversial due to the risk of priapism, the risk is outweighed because it helps to reduce anxiety and improves handling and anaesthetic induction. After this, the colt is then sedated with an α-2 adrenoceptor agonist (such as romifidine) with butorphanol. It is important to increase the dose if the animal is fractious because an adequate level of sedation is required before induction of anaesthesia.

Before induction, palpation of the scrotum and inguinal region is performed to ensure that both testes are descended and that there is no scrotal hernia. Anaesthesia is normally induced with ketamine, and the horse is then positioned in dorsal recumbency. It is often helpful for an assistant to straddle the horse across its thorax while holding the forelimbs to balance the horse in dorsal decumbency. Lignocaine HCl (20 ml) is injected into each testis, and a further 10 ml is injected subcutaneously into the scrotal midline. The scrotum is aseptically prepared for surgery, and a fenestrated drape is placed.

The surgeon kneels on the colt's right side. The left testis is grasped with the left hand and pushed against the scrotum; the median raphae of the scrotum are readily identified as a distinct line. A skin incision parallel to the median raphe is made over the left testis, 1 to 5 cm from midline and of appropriate length, depending on the size of testicle. A second incision is then made in the vaginal tunic and the testis exteriorised. Traction is applied to the testis to tense the epididymal ligament, which is then sectioned with a scalpel. Forceps (Lane, Aliss, or Babcock) are applied to the vaginal tunic, and it is bluntly separated from the cord and spermatic vessel by traction. Serra emasculators are then applied to the vessels and ductus deferens and the testis removed. The emasculators are left in place for 3 to 4 minutes depending on the size of the colt. After removal of the emasculators, the spermatic vessels are inspected to ensure adequate haemostasis. A swab is then used to bluntly dissect the vaginal tunic from the associated scrotal connective tissue to the level of the external inguinal ring. The amount and texture of connective tissue varies considerably between breeds and types of horses, often being substantial in native ponies and cobs. The vaginal tunic is again grasped with forceps at the site of the incision and twisted to ablate the lumen down to the level of the external inguinal ring. The forceps are handed to an assistant to apply traction as the surgeon places a transfixing ligature of 5 M Polyglactin 910 through the vaginal tunic at the level of the external inguinal ring; this is hand tied on either side of the fixation. The emasculator is then applied to the vaginal tunic distal to the transfixing ligature, and it is removed; the emasculators should be left in position for 2 minutes to ensure adequate haemostasis of the cremaster muscle vasculature. The process is repeated on the right side, although it is quicker to remove both testes first, leaving emasculators attached on the right testis while closing the left vaginal tunic.

After removal of both testes, the two parallel scrotal incisions are joined, and the median raphe of the scrotum is dissected out with scissors. This provides adequate drainage of the subcuticular dead space. The wound is inspected and the scrotum stretched to ensure that no connective tissue can prolapse through the incision; any tissue that may do so is removed. The horse is placed in lateral recumbency for recovery. As noted previously, this is the routine procedure used by the authors; however, in many circumstances, particularly when there is greater risk of wound contamination, the surgeon may elect to perform a closed castration whereby the vaginal tunic is not opened.

Postoperative management is as for standing castration.

Theatre-Based Castration

This technique, although the ideal procedure, is usually reserved for mature stallions due to cost. The aim of the surgery is to ensure a high level of sterility during the procedure and to close the skin incision after completion of the castration.

There are some variations in technique depending on the preferences of the surgeon (Kummer et al. 2009); the authors recommend the following technique.

Under routine general anaesthesia the urethra is catheterised, and the prepuce is temporarily closed with a continuous suture of 3 M nylon. The scrotum and inguinal region is aseptically prepared for surgery. A disposable impervious drape is applied, and an approximate V-shaped section of the drape is excised to expose the scrotum and the inguinal region while leaving the prepuce covered. An iodine impregnated adherent drape is then applied. A parainguinal incision is made midway between the external inguinal ring and the scrotum; care is taken to avoid large subcuticular vessels. The subcuticular fascia is incised, and then blunt dissection is used to expose the vaginal tunic. The tunic is grasped with Allis forceps, selecting a site where the cremaster muscle is not attached. The tunic is excised using a scalpel, and the incision extended with scissors to allow extraction of the testis by finger traction; the ligament of the epididymis is then transected. The ductus deferens and testicular vessels are ligated using a single 5 M polyglactin 910 suture. The spermatic cord and associated vessels are then severed with an emasculator distal to the ligature. A 'graft passer' is used to place a ligature around the vaginal tunic close to the external inguinal ring; this technique allows for minimal dissection, reduced chance of haemorrhage, and smaller dead space. The subcutaneous tissues are closed with 3 M polyglactin 910, and the skin with 2 M polyglactin 910 placed intradermally. The process is repeated on the contralateral testicle.

After recovery from general anaesthesia, postoperative management for the horse is as described later in this chapter for standing castration, apart from the use of antibiotic medication which is not required because of the high level of asepsis.

Laparoscopic Castration

Routine castration can be undertaken laparoscopically in the standing horse by ligating and transecting the vasculature and ductus deferens intraabdominally. In most animals the testes will undergo avascular necrosis. However, in 3.4% of stallions, a testis does not become necrotic due to the establishment of an alternative blood supply from the cremaster muscle and/or the external pudendal artery (Voermans et al. 2006). Laparoscopic techniques have also been combined with open surgery (Rijkenhuizen & van der Harst 2017).

Complications

Eventration

The most common severe complication of equine castration is eventration, the protrusion of small intestine through the inguinal canal (so called 'indirect herniation'). This can lead to trauma of the exposed bowel and is rapidly fatal. Eventration is prevented by techniques that either do not open the vaginal tunic or open it but after removal of the testis is subsequently close (as described previously). The risk period for herniation is within the first 36 hours of surgery. When eventration occurs, it is not possible to replace the herniated loop of small intestine into the abdomen. Therefore initial treatment requires protection of the exposed bowel and prevention of further herniation. If there is a relatively short

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loop of small intestine involved, it can be treated as an essentially 'first aid' measure by just suturing the scrotum to contain it in order to facilitate transport of the affected individual to a surgical facility. If the loop of exposed bowel is substantial and/or it is damaged, immediate field-based general anaesthesia may be required to facilitate replacing the exposed intestine into the scrotum and retaining it by suturing the scrotal incision. Sometimes resection of the traumatised intestine is required, and the bowel clamps on the transected stumps are then sutured within the scrotum to enable transport for surgery. All cases will require general anaesthesia and a laparotomy to enable hernia reduction and facilitate resection and anastomosis of the affected bowel. The prognosis is good in peracute cases; however, in chronic cases, cardiovascular compromise and endotoxaemia will reduce the prognosis.

Herniation of omentum is a much less serious complication, and generally the only indication is presence of the omentum protruding from the scrotal incision with no other clinical signs. Treatment involves applying gentle traction to expose a section of clean omentum, then simply excising the exposed tissue. If the amount of tissue is large, then ligation should be undertaken first; it is prudent to undertake a rectal examination to palpate the internal inguinal ring and thus ensure that no bowel appears to be entrapped. In simple cases the prognosis is good.

Haemorrhage

Bleeding from the scrotum, cremaster muscle and subcuticular vessels is generally self-limiting and not significant, although it can be alarming to owners.

Bleeding from the testicular vessels is potentially serious. This can be common particularly after standing castration, especially when ligation has not been performed. Many clinicians regard the presence of blood dripping from the scrotum for up to 24 hours after surgery as acceptable, but it can be avoided. Minimal but persistent bleeding is frustrating for the veterinarian because it may result in owners requesting often unnecessary repeat visits. Generally, this can be avoided by placing good quality emasculators correctly and leaving the emasculators in place for at least 5 minutes as described previously.

If the bleeding is continuous rather than intermittent dripping or has persisted for over 24 hours, then ligation may be necessary. The cardiovascular status of the horse should be assessed clinically and a packed cell volume (PCV) value obtained before treatment.

In cases with cardiovascular compromise or if the bleeding is minimal, the scrotum can be packed with gauze swabs in an attempt to apply pressure to the stumps of the cords; these can be sutured in place with heavy catgut. The gauze is removed 2 days later by breaking the catgut suture by a sudden pull on the gauze (Schumacher 2012).

In cases in which there is severe haemorrhage without cardiovascular compromise or corrected haemorrhagic shock, short general anaesthesia may be required. The stump of the cord can be located and religated using a 5 M polyglactin 910 ligature and repeat emasculation. In addition, gauzes can be sutured in place with heavy catgut as described previously.

Intraabdominal haemorrhage has been anecdotally reported postcastration; occasionally there is continued bleeding despite repeated attempts to ligate the stump. In such instances, standing laparoscopic ligation may be indicated (Trumble 2000). Using the author's field anaesthesia technique (described previously), the vaginal tunic is ablated by twisting. The author has used this technique with concurrent laparoscopic visualisation of the internal inguinal ring; the spermatic cord stump does not retract into the abdomen. Because it is not present in the resected vaginal tunic, the stump must remain in the region of the external inguinal ring. In the authors' experience, intraabdominal haemorrhage has not occurred using this technique.

Infection

Bacterial infection most frequently results from contamination of the scrotal wound after surgery and therefore cannot be prevented at the time of surgery. Attempts should be made to reduce contamination, especially in the first 36 hours postoperative, when the animal is confined; the bedding must be clean, of adequate depth, and a faeces removed frequently. Straw or paper bedding is preferable to shavings, especially in young animals that are more likely to lie down; deep litter systems are not appropriate. After this period, increased exercise is important to reduce swelling and oedema and to promote drainage. In young animals, free paddock exercise is appropriate. Consideration of the timing of surgery is important as in summer months fly-borne contamination is difficult to prevent. In winter, very low temperatures can hamper scrotal healing. In older horses, controlled exercise may be more appropriate; hand walking, a treadmill, walker exercise, or leading are all suitable. A significant period of walking exercise is recommended: 45 minutes three times daily. Some trainers favour swimming during this period; although this is very effective at reducing swelling and oedema, it cannot be recommended due to the potential risk of an ascending infection through the inguinal canal and subsequent peritonitis.

Scrotal Infection

In techniques without scrotal closure, subcutaneous scrotal infection is self-limiting because in most cases there is adequate drainage. Removal of the median raphe of the scrotum (as described previously) to create a single subcutaneous space further enhances drainage.

If the scrotum is closed, contamination of the sutures may result in bacteria tracking into the subcutaneous dead space and the creation of a scrotal abscess. If scrotal drainage is inadequate in open scrotal techniques, the skin may heal and trap infection subcutaneously, again resulting in a scrotal abscess.

Treatment primarily depends on establishing adequate drainage; with the horse adequately restrained, sutures must be removed and the scrotal incisions reopened. Local anaesthetic is injected into the abscess before reopening the incision, and this can almost always be achieved by blunt dissection, either digitally or using scissors. In extreme cases, general anaesthesia and surgical excision of the scrotum and removal of the median raphe may be appropriate. Exercise to reduce swelling and oedema is important; animals turned out may be inactive due to pain caused by the infection. Nonsteroidal antiinflammatory medication is important to reduce pain and swelling and facilitate exercise. Bacterial culture and sensitivity is appropriate but not always necessary. Broad spectrum antibiotics should be administered, although culture and sensitivity should be considered in serious cases in which longer course antimicrobial therapy may be necessary.

Scirrhous Cord

Chronic micro pyogranulomatous infection of the stump of the spermatic cord is a more serious complication caused by bacterial infection. The source of infection is either contamination at surgery or, more commonly, secondary to scrotal infection. Techniques that do not close the vaginal tunic increase the risk of the development of a scirrhous cord secondary to scrotal infection. Horses with this condition will have a chronic purulent discharge from the scrotum.

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Palpation reveals a firm thickening of one or both of the spermatic cord stumps. Because the infection is pyogranulomatous with multiple microabscessation, antibiotic treatment alone is unsuccessful and inappropriate. Appropriate treatment is by surgical resection, performed under general anaesthesia. There is frequently substantial highly vascular fibrosis and adhesion formation around the stump, which needs to be dissected by both sharp and blunt techniques from the surrounding tissues. The spermatic stump is freed up to the level of the external inguinal ring and then transected with emasculators at the level of the external inguinal ring; in most cases, this is proximal to the thickened infected stump. However, on rare occasions the thickened stump continues into the inguinal ring, in which case laparoscopy may be indicated to facilitate transection of the stump proximal to the infection. Despite the potential risk of peritonitis, in the authors' experience, this is extremely rare. The scrotum is left open for drainage. Appropriate antibiotics are administered based on culture and sensitivity, and the open scrotal wound is managed with appropriate exercise and frequent lavage. The prognosis with appropriate treatment is excellent; however, it is important to emphasise that even in cases in which there are financial constraints on the cost of treatment, surgery is necessary because attempts to treat with antibiotics alone will be unsuccessful and merely add to the cost.

Peritonitis

Peritonitis is a rare but serious complication of equine castration. Animals presenting with any of the following signs postoperatively should be considered at risk: pyrexia, inappetence, colic, lethargy, and/or weight loss. Diagnosis is made after the analysis of peritoneal fluid obtained by abdominocentesis. Gross examination reveals a turbid and cloudy fluid. Laboratory investigations usually demonstrate an elevated total nucleated cell count and elevated protein concentration, and in a cytological examination, there are degenerate neutrophils and bacteria. The sample should be submitted for culture and sensitivity. Aggressive antimicrobial therapy, along with appropriate management of the scrotal wound/scrotal abscess and/or scirrhous cord is required; in severe cases, peritoneal lavage may be necessary. The prognosis with appropriate management is good, although adhesion formation as a cause of colic after castration-induced peritonitis has been observed.

Postoperative Swelling and Oedema

Swelling and oedema of the scrotum and prepuce are commonly seen over the first 5 days after castration but generally resolve by 2 weeks. An appropriate exercise regime should help to prevent the early closure of the surgical incision and thereby reduce swelling. Swelling that is severe or persists beyond this period should increase the suspicion of the presence of infection (see previous mention).

Cryptorchid Surgery in the Horse

Aetiology

Failure of one or both testes to descend into the scrotum is relatively common in the horse. The retained testis or testes may be present within the inguinal ring or in the abdomen. In some cases the gubernaculum and/or epididymis are present within the inguinal canal, although the testis/testes remain within the abdomen. Abdominal testes are most frequently located close to the internal inguinal ring but may be situated anywhere between the inguinal ring and the kidney.

It is noteworthy that anorchidism (congenital absence of the testes) and monorchidism (only a single testis present) are extremely rare, and so most cases seen in primary care practice will be either unilateral or bilateral cryptorchids.

Investigation of Cryptorchidism

Careful palpation of the inguinal region under sedation may reveal the presence of an inguinal testis. Ultrasonography undertaken at the level of the inguinal ring enables an inguinal testis or an abdominal testis positioned close to the internal inguinal ring to be identified. Rectal palpation of the inguinal region may also be helpful.

In horses of unknown history that exhibit stallion-like behaviour but in which no scrotal testes can be palpated, endocrine investigations are required to confirm the presence or absence of retained testes. This can be undertaken using an hCG stimulation test (in which elevated testosterone concentrations after administration is diagnostic) or measurement of basal oestrone sulphate (in which elevated concentrations are diagnostic). In these cases, careful visual examination of the scrotum for scarring from previous surgery may also be helpful.

Surgical Technique

Cryptorchidectomy can be carried out by laparotomy, using an inguinal, parainguinal, suprapubic midline, or flank approach. Laparoscopic cryptorchidectomy is widely used in hospital practice; however, recent studies have found an increased complication rate compared with traditional techniques (Cribb et al. 2015).

Inguinal Exploration

In cases in which an inguinal testis can be palpated or identified on ultrasound examination, surgical inguinal exploration is indicated. This can be done with the animal standing after administration of local anaesthetic into the scrotal and inguinal regions but is easier under general anaesthesia.

After routine aseptic skin preparation, a scrotal incision is made parallel to the midline. Blunt finger dissection is used to expose the inguinal region. The gubernaculum and/or the epididymis and/or the testis may be palpable. If not, then direct visualisation may reveal a white structure with a smoother, almost shiny, appearance compared with the local fatty connective tissue. This tissue is likely to be the gubernaculum and can be grasped with appropriate Lane, Babcock, or Allis forceps. Traction on the gubernaculum will often deliver the inguinal testicle into the surgical incision, and a routine castration can then be performed. Care should be taken to ensure that a testis is present because occasionally only the epididymis can be exteriorised because the testis is too large to pass through the internal inguinal ring and remains in the abdomen; rarely the epididymis is mistaken for a testis and is removed, thus leaving the testis *in situ* within the abdomen.

Parainguinal Exploration

If the testis is abdominal or inguinal, a parainguinal approach is preferred. In bilateral abdominal cryptorchids, both testes can usually be retrieved from a unilateral parainguinal incision. Rarely, bilateral parainguinal incisions may be required for bilateral abdominal cryptorchids; this is still preferred to a midline approach.

A skin incision is made centred on the cranial boarder of the inguinal ring, 3 to 5 cm from the midline. Using blunt dissection, it is continued through the subcutaneous tissue to expose the

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aponeurosis of the external abdominal oblique muscle, taking care to avoid damage to any blood vessels. This fibrous structure is incised with a scalpel, then the internal oblique and rectus muscle fibres are separated by blunt dissection between their fibres to produce a grid approach, which exposes the peritoneum. The peritoneum is then penetrated with a finger. The incision only needs to be large enough to allow access of two fingers. The first and index fingers are then placed through the incision and angled laterally towards the internal ring. The abdominal testis can frequently be grasped between the fingers and exteriorised by traction. If unsuccessful, the incision can be extended to allow the insertion of a hand and, if necessary, an arm into the abdomen. A systematic search of the abdomen is then undertaken starting at the internal inguinal ring to confirm the absence of any structure including a previously severed spermatic cord passing into the inguinal ring. The examination should then logically progress towards the bladder neck, along the bladder, and up to the kidney. Occasionally, an abdominal testis is situated caudal to the inguinal ring and is found within the pelvis. Familiarity with the feel of the tubular nature of the epididymis and more homogeneous and slightly firmer texture of the testis, compared with the abdominal viscera, is helpful. Rarely, the testis is not identified using this approach; one of the authors' first surgical mentors then claimed 'if all else fails opening the palm of the hand in the abdomen and *thinking testis* sometimes results in the offending structure being identified'.

In bilateral cases, both testes can usually be removed from a single parainguinal incision; occasionally bilateral incisions are necessary when the spermatic cord is too short on the contralateral side. A midline approach is not recommended as the spermatic cord may be too short to enable the testis to be exteriorised. A parapenile midline approach can be used in bilateral cases but requires more dissection than the parainguinal approach. A direct approach through the inguinal ring is not recommended due to the increased risk of subsequent inguinal herniation.

After emasculation and removal of the testis, the wound is closed with a single suture placed through both deep muscle layers to close the grid, followed by simple interrupted closure of the aponeurosis, using 5 M polyglycolic acid 910. The subcutaneous tissue is closed with 3.5 M polyglycolic acid 910, followed by 3 M polyglycolic acid 910 intradermal sutures.

Laparoscopic Exploration

Ultrasonographic examination of the abdomen is very important before attempting laparoscopy because it often enables the identification of the approximate location of the testes and therefore assists with planning of the laparoscopic approach.

Standing Laparoscopy

Standing laparoscopic cryptorchidectomy is a very elegant technique for the well-behaved and easily handled mature horses (Hendrickson & Wilson 1997). It is performed via two paralumbar portals, employing similar techniques to those used for ovariectomy (see later in this chapter). In bilateral cryptorchid cases the procedure can be repeated on the contralateral side. In unilateral cases, castration of the descended testis can be carried out at the same time, although ligation and transaction of the spermatic cord and vessels can still be done laparoscopically (Sassot et al. 2017).

Laparoscopy Under General Anaesthesia

Laparoscopy under general anaesthesia may be appropriate for young and unhandled horses and ponies. An operating table capable of tilting into the Tredelenburg position is required. The laparoscope portal is placed cranial to the umbilicus; two instrument portals are placed either ipsilaterally or contralaterally in the caudal abdomen. After insufflation of the abdomen, the table is elevated into the Tredelenburg position so that the parts of the colon are displaced cranially to reveal the inguinal rings. Abdominal testes can be removed after transaction and ligation using a 'ligasure', via extension of the laparoscopic incision.

Laparoscopy Complications

The most serious complication of the laparoscopic castration is penetration of viscera, when inserting the laparoscopic and instrument portals; this risk can be minimised as described for laparoscopic ovariectomy (see later in this chapter). In a recent review (Crib et al. 2015), it was found that horses undergoing laparoscopic cryptorchidectomy had increased surgical preparation times, increased surgery and anaesthesia times, and greater postoperative complications, compared with horses undergoing open cryptorchidectomy. Laparoscopy may be advantageous for a second attempt at cryptorchidectomy or if the testis location is unknown before surgery (Crib et al. 2015).

Castration (Ovariectomy of the Female (Mare) Horse

Indications

Routine ovariectomy of the mare is indicated to reduce unwanted 'mare-like' behaviour and prevent oestrus. It is likely that prevention of inappropriate aggressive and dominant behaviour is most effective if ovariectomy is carried out in young mares soon after the behaviour becomes apparent. However, the effects of ovariectomy on mare behaviour have not yet been fully investigated. Prepubertal ovariectomy of mares to prevent inappropriate behaviour is not currently undertaken, and its effectiveness has not been investigated. Ovariectomy is also indicated in cases of ovarian pathology, most commonly a granulosa cell tumour in which the mare may demonstrate a broad range of clinical signs, and there is commonly substantial enlargement of the ovary.

Historical Perspectives

In the late 19th and early part of the 20th century, ovariectomy of mares was commonly performed in France and the UK; it was routine in the British military. The surgical technique employed was a transvaginal colpotomy, using chain ecraseurs to transect the ovarian pedicle and oviduct. Ironically, this was in some way an extremely modern technique, performed in the standing horse via a natural orifice approach. The practice fell out of favour as the horse became less essential for the armed forces. As the availability of hormonal methods of controlling ovarian activity increased and because simple suitable routine surgical methods were not applicable, there was a decline of routine mare ovariectomy by the mid to late 20th century. Nowadays, particularly in sports horse competitions, the gelding rather than the stallion or mare is preferred, as most riders regard the former as more predictable and trainable. Consequently, ovariectomy of fillies and mares appears to be becoming more popular (despite the legalisation of progesterone therapy to suppress oestrus for mares under FEI regulations). Recently, several minimally invasive laparoscopic techniques, either via a flank or vaginal approach, have been described. In the sport and leisure horse, the practice is gradually becoming more popular.

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However, evaluation of the benefits of mare ovariectomy and the most appropriate timing of the procedure need to be investigated so that mare owners can be advised correctly.

Surgical Technique

Routine Ovariectomy

A variety of techniques for ovariectomy are currently in use. The most common is a laparoscopic technique using three portals per side. The ovarian pedicle is sealed and transected using a ligature; the ovary is then removed by joining the two instrument portals using a blunt grid approach through the abdominal muscles. This results in the creation of a 20 cm long flank wound. Colpotomy techniques have also been reported; the challenge with using this technique is ensuring adequate sterility and closure of the vaginal incision. There have been other approaches advocated, including pararectal, hand-assisted laparoscopic, and trochar techniques.

In the authors' practice, a laparoscopic technique using a morcelator (Lund et al. 2014) is used. This involves two laparoscopic portals on each side of the animal. The ovarian pedicle is anaesthetised by the injection of lignocaine, and then the pedicle is sealed and transected using an electrosurgical ligating and cutting device, in a caudal to cranial direction, leaving the ovary suspended by the final 5 mm of cranial ovarian pedicle. The morcelater is then introduced through the instrument portal, and the ovary is removed. The two paired portals are closed with absorbable sutures in the muscle, and intradermal skin sutures. The mare can resume exercise 10 days later. It is likely that, with the continued development of safe elegant cost-effective techniques, routine ovariectomy of mares will once again become a commonly performed procedure in equine hospital practice.

Removal of Granulosa Cell Tumours

Granulosa cell tumours are benign ovarian tumours; they vary in size from little more than that of a normal ovary to over 50 cm in diameter in the most extreme cases. Removal by laparoscopic transection and ligation using an electrosurgical sealing and cutting device in the standing horse, as described previously, is now routine. Alternatively, laparoscopic stapling devices or ligatures can be used.

Removal of the ovary can be undertaken in several ways. The technique used should be selected based on three factors: the size of the mare, the size of the ovary, and the depth of extra peritoneal fat. Granulosa cell tumours are multilocular and fluid filled; therefore the size of tumour can be significantly reduced by aspiration of the fluid. Relatively small tumours, especially in large lean mares, can be removed via a flank incision. The instrument portals are connected, and the skin incision is enlarged to an appropriate size for the ovary. The fascia and external oblique muscles are transected, and the incision is enlarged using a blunt finger grid dissection through the internal oblique and the transverse muscles; the peritoneum is also bluntly opened. The ovary is elevated into the incision by traction on the grasping forceps. With a portion of the tumour exposed, a stab incision is made into it, which enables a suction catheter to be introduced so that fluid can be aspirated to reduce the tumour size. Once flaccid, the mass can be exteriorised through the flank incision, and the wound is then closed. In all but the largest wounds, closure of the deeper layers is not necessary. The external oblique and fascia is closed using a simple continuous pattern with 5 M polyglycolic acid 910; the subcuticular layer is closed using a simple continuous pattern with 3 M polyglycolic acid 910, followed by intradermal sutures using 2 M polyglycolic acid 910. Alternative techniques for flank removal include intraabdominal needle aspiration and the use of endobags, with and without morcellation.

For large or moderate sized tumours in small or obese horses and ponies, it is preferable to remove the mass in a second procedure under general anaesthesia. With the horse or pony in dorsal recumbency, a midline laparotomy incision is made, at least large enough for the insertion of the surgeon's arm. A 50 × 50 cm disposable drape is placed into the abdomen. The ovary is placed onto the drape, and the drape carefully opened out. The four corners of the drape are then grasped by the surgeon and pulled out of the incision so that the ovary is pulled up into the incision. A stab incision is then made in the ovary to enable a suction catheter to be pushed into the mass to aspirate as much fluid as possible. When the mass is more flaccid, it is exteriorised through the incision by traction on the drape; the celiotomy incision can be extended if necessary. The wound is then closed routinely, and the horse recovered.

Complications

Before the advent of laparoscopic techniques, the complication rate after open surgery was high. This is because the ovarian pedicle is relatively short and difficult to access, thus increasing the potential for inadequate ligation of the pedicle, leading to a high risk of potentially fatal haemorrhage. Traction on the ovary causes pain, which often necessitates increasing the depth of anaesthesia, and thus possible anaesthesia complications. Current laparoscopic techniques have resolved these issues. The pedicle is routinely anaesthetised by visually directly injecting local anaesthetic and then effectively ligated without the use of excessive tension. The main complication results from the risk of inadvertent visceral or splenic penetration when placing the first laparoscopic portal. This can be reduced by careful preoperative planning, including rectal palpation to assess the position of the colon, spleen, and caecum. The introduction of air into the abdomen via a Varres needle or teat cannula before the placement of the first trochar and cannula is also helpful. Guarded sharp trocars are also frequently used and are quite satisfactory. However, the threaded trocar further reduces the risk of visceral penetration as there is no forward stabbing force required for its insertion, and the peritoneum is incised in parallel plane. In most cases of inadvertent caecum or large colon penetration, the cannula remains in the viscera. This enables the surgeon to extend the incision so that the bowel and cannula can be carefully exposed and elevated by placing stay sutures either side of the penetration. The abdomen can then be packed-off with sterile saline–soaked, gauze swabs. The cannula can then be removed from the bowel, and the incision closed with a continuous double layer of inverting sutures, with 2 M polyglycolic acid 910 on a round body needle. If the bowel separates from the trocar, then significant faecal peritoneal contamination will occur, leading to rapidly fatal peritonitis. Conversion to a ventral midline laparotomy under general anaesthesia may be required to facilitate closure of the colon penetration followed by abdominal lavage.

Large flank incisions are associated with higher morbidity rates due to seroma formation; they are also unsightly and may leave significant scarring. The complication rate is very low for an elective midline laparotomy. This technique should always be considered when the tumour is large, especially in small or fat animals, and when a good postoperative cosmetic result is important, such as in show animals.

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PART 4

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22

Infertility in the Cow Due to Functional and Management Deficiencies

TIMOTHY J. PARKINSON

General Considerations

Fertility is one of the key determinants of the lifetime performance of a cow. For beef cows and for pastoral dairy cows, it is necessary for a calf to be produced every 365 days. For intensively managed dairy cows, which can maintain long periods of high lactational yield, the need to produce a calf each year is less of an imperative (Dijkhuizen et al. 1985); yet, even for these animals, regular calving is necessary for the establishment of lactation and breeding of herd replacements.

Regular breeding depends on the normal function of the reproductive system. In order to breed regularly, the cow has to have functional ovaries, display oestrus behaviour, mate, conceive, sustain the embryo through gestation, calve and resume oestrous cycles, and restore uterine function after calving. Management, disease, and the genetic makeup of the animal can affect each of these aspects of reproductive function. Sterility, meaning an absolute inability to reproduce, is rare, but reduced fertility, referred to as subfertility, is common.

Prevalence and Cost of Infertility

Prevalence

Evidence for the decline in fertility of high yielding dairy cows has been steadily accumulating over about the last 50 years. Over that period, first service conception rates and pregnancy rates have decreased, and intercalving intervals and the proportion of cows culled for infertility has increased.

The decline in first service conception rates has been extensively reviewed. For example, Butler (1998) showed a decrease of first service conception rate from 65% to 40% between 1951 and 1996. Lucy (2001) and Mann (2004; Fig. 22.1) showed similar trends in the literature over the same period. First service conception rates of 40% to 45% (e.g., Bousquet et al. 2004) and pregnancy rates (defined as number of cows pregnant/number of insemination) of 27% (Cook & Green 2016) have been reported. Dubuc and Denis-Robichaud (2017) reported first service conception rates of (25th quartile) 24%, (50th quartile) 40%, and (75th quartile) 45% for lactating Holstein cows in Canada.

This decline of conception and pregnancy rates has been reflected in changes in culling rates. In the 1950s and 1960s, culling rates for infertility were around 5% (e.g., Gracey 1960, Leech et al. 1960). Today the figure is around 20% in the UK (Forbes 2000, Esslemont 2003), with similar rates reported from North America (Milian-Suazo et al. 1988, Ribeiro et al. 2003) and Europe (Frerking 2003, Bagnato 2004). Adamczyk et al. (2017) reported that 40% of Polish Holstein–Friesians were culled for infertility and reproduction problems. Involuntary culling for failure to conceive and other fertility-related culling is one of the most common causes of removal of cows from dairy herds (other important causes of culling are for lameness and mastitis) (Gardner et al. 1990, Miller & Dorn 1990, Forbes 2000) and, in consequence, there is relatively little opportunity to cull for other production-related traits. Unfortunately, not all cows that are culled as being 'infertile' actually are; abattoir surveys of cows that have ostensibly been culled for fertility consistently record that a significant proportion of animals are pregnant (Singleton & Dobson 1995, Sheldon & Dobson 2003), sometimes even in the third trimester of pregnancy.

Likewise, calving interval has increased over a similar period. Hare et al. (2006) showed a progressive increase in calving intervals for first parity (Fig. 22.2) and second parity Holsteins in the US. Similar trends were shown for other major dairy breeds. In the UK the average calving interval for Holstein–Friesians was reported (Gates 2013) as 428 days, with the figures for the 75th quartile and 90th decile being 463 and 546 days, respectively.

Infertility is a considerable problem in beef herds, although relatively few data exist detailing its prevalence. Nevertheless, postpartum and lactational anoestrus are significant problems in beef herds, which, if animals are culled on the basis of calving pattern, can lead to significant losses of animals due to conception failure. New Zealand data indicates that between 7% and 11% of beef cows fail to conceive (Morris & Cullen 1998). Under the range conditions of northern Australia, 75% pregnancy rates are frequently achieved, although this can drop to as low as 15% (O'Rourke et al. 1991). However, for feedlot and intensively managed beef cattle, pregnancy rates of up to 98% have been reported (e.g., Warren et al. 1988), with first service conception rates of between about 75% and 80% (Brown et al. 1991, Mann et al. 1998).

Economic Consequences of Infertility

Bozworth et al. (1972) stated that 'infertility is one of the important economic losses in high producing herds and…modern feeding and management practices in large herds may accentuate the problem'. This comment is equally true today.

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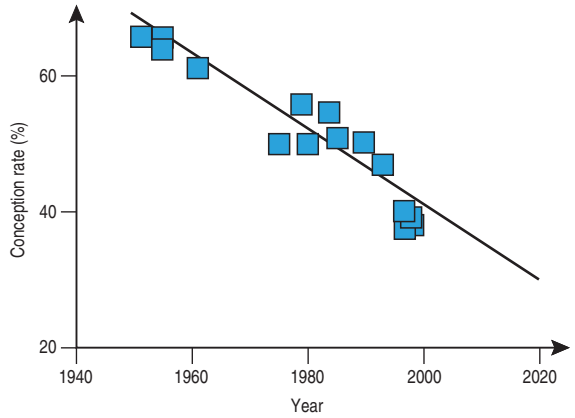
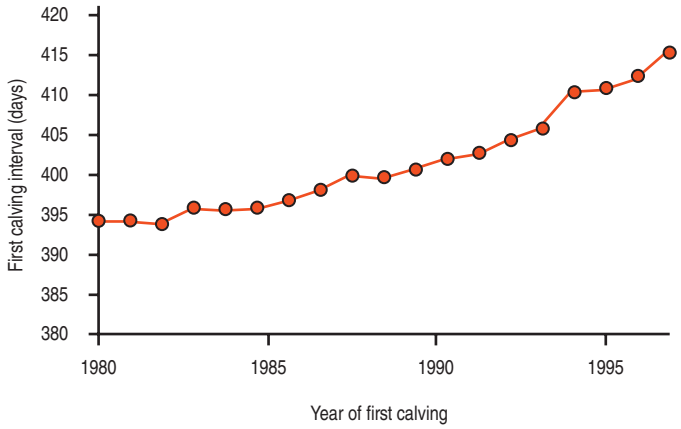


• **Fig. 22.1** Changes in conception rate of cattle in the literature between 1950 and 2020. (Adapted from Mann 2004.)

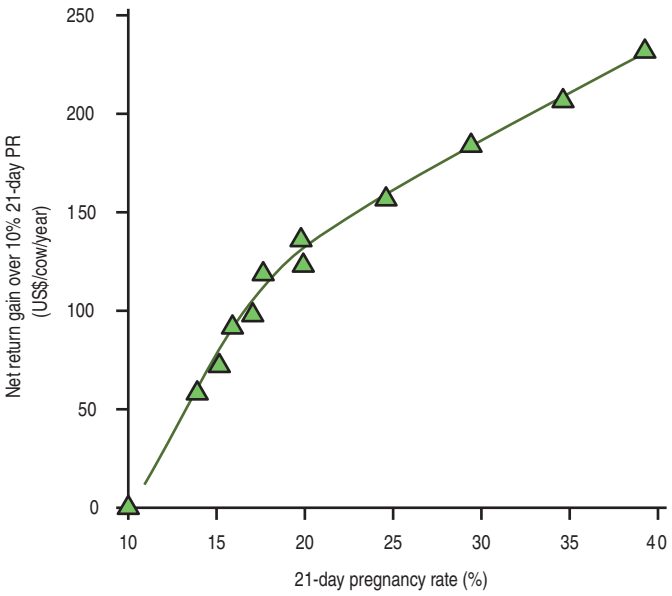


• **Fig. 22.2** Trend in calving interval between first and second parities by year of first calving for Holstein cows. (Adapted from Hare et al. 2006.)

Studies of the consequences of the losses due to infertility among dairy herds have attempted to quantify its effects on production and financial performance. All agree that infertility is expensive, although also highly dependent on the production system. Infertility leads to a loss of milk production, a loss of income from calf sales, and an increase in the replacement rate of cows with first-calving heifers. Its effects may, to some extent be mitigated by the income that is derived from cull animals, although this mitigation is largely a matter of perception rather than actuality because the costs of replacing mature cows with first-calving heifers are substantial.

In the early 1990s, US data suggested that the total cost of breeding problems was $24 per cow per year, compared with $36 for mastitis (Kaneene & Hurd 1990). In the UK, Esslemont (1992) calculated that for dairy herds, at 1992 prices of milk, feed, calves, replacement heifers, culled cows, and so forth, the cost to the farmer for each day's extension of the calving interval beyond 365 days was approximately £3.50. Estimates from the US also suggested that a 1% increase in conception rate was worth up to approximately $7.50 per cow per year (Pecsok et al. 1994a). Improvements in oestrus detection rates have also been associated with improved herd economic performance: Pecsok et al. (1994b) considering that an improvement from 60% to 70% detection efficiency was worth $6 per cow per year.

By the early 2000s an estimate of the costs of subfertility to the UK dairy herd (Esslemont 2003) had increased substantially to around £180 for every cow in the national herd. More recently,



• **Fig. 22.3** Approximate economic net return gain (US$/cow/per year) of improving reproductive efficiency by 10 percentage points of a 21-day pregnancy rate (21-d PR). (Based on Cabrera 2014.)

Cattaneo et al. (2015) estimated costs of infertility in Argentine dairy cows as being US$183, $358, $520, and $770 per cow per lactation for involuntary days open periods of 0 to 30, 30 to 60, 60 to 90, and 90 to 120 days, respectively. Laven (2017) suggested that the cost of culling a cow due to poor fertility was between £1000 and £1200, resulting in a net loss of £13,000 to a 100–cow farm with an involuntary culling rate of 19%. Cabrera (2014) examined the costs of infertility in cows in TMR systems and related the proportion of cows that conceive per 21-day period (21-day pregnancy rate) to the economic performance of the herd (Fig. 22.3): increasing the pregnancy rate by one percentage point increased the economic net return (US$/cow per year) by $14, $10, $7, $5, $4, and $3 when the 21-day pregnancy rate increased within 10% to 15%, 15% to 20%, 20% to 25%, 25% to 30%, 30% to 35%, and 35% to 40%, respectively. Similarly, Shalloo et al. (2014) estimated the costs of increasing replacement rate in a pasture-based dairying system, showing that farm profitability decreased by €6840 and €12,470 for a 100–cow herd when replacement rate increased from 18% to 23% and 23% to 27%, respectively; each 1-day increase in mean herd calving date reduced profitability by €3.86/cow/day.

Various tools have been devised to give farmers a better understanding of the costs of fertility. For example, Kossaibati and Esslemont (1997) developed the Fertex Score, which places an economic value on various aspects of reproductive performance such as days open, involuntary culling services per conception, and the effects of changing voluntary wait periods. Similarly, the Australian InCalf programme (InCalf 2000) also identified the areas in which the greatest losses due to infertility occur.

Overview of the Causes of Infertility

Domestic cattle are not a particularly fertile species. Per-service conception rates are rarely over 55%, and in dairy cattle they can be very much lower than that. Whether this is a consequence of domestication has been widely debated; certainly, wild Bovidae have much better fertility than domestic cattle, but they are also

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under much less demand for production. For domestic cattle, there are many factors that impinge upon fertility. These include the following:

- Congenital and acquired lesions of the genital tract (structural causes)
- Ovarian dysfunction
  - anoestrus
  - expression of oestrus
  - follicular or luteal dysfunction
  - age- and breed-related effects
- Stockmanship and management
  - oestrus detection
  - nutritional management
- Infections
  - metritis complex/retained fetal membranes (Chapter 23)
  - venereal diseases (trichomonosis, campylobacteriosis, ureaplasmosis; Chapter 24)

The most serious impairment of fertility is seen in dairy cows. There is now no doubt that reproduction is adversely affected by high yield, as a result of metabolic stress, feed imbalances, and negative genetic relationships between yield and reproduction. As a result, nearly all aspects of dairy cattle reproduction are affected, ranging from the expression of oestrus, to mechanisms related to follicular and luteal function, to uterine environment, to embryonic development. Concurrently, the stockmanship of the labour that is available to dairy farms is declining throughout much of the developed world. In consequence, oestrus detection rates are declining, often necessitating the use of automated detection systems or pharmacological interventions to maintain acceptable conception rates.

One of the consequences of the decline in fertility of dairy cows is the realisation that, for a dairy industry to be sustainable, fertility must be maintained at an acceptable level. This has led to the recognition that because fertility has declined through a negative genetic relationship with selection for milk yield, the potential exists for it to be improved though genetic selection. Hence, many cattle-breeding organisations are now including breeding values for fertility and longevity alongside yield, which appears to be halting (or perhaps even starting to reverse) the decline in the genetic components of fertility. It has also created opportunities for veterinarians to work alongside the dairy industry, not just to apply 'quick fixes' for subfertility but to become more deeply involved in the management of fertility (with consequent involvement in feeding, housing, labour skills, etc.), in order to ensure that fertility is maintained at a level at which the industry itself is sustainable.

Structural Causes of Infertility in the Individual Cow

Both congenital and acquired abnormalities of the genital system can influence fertility. Congenital abnormalities are much less common than those that are acquired; Kessy (1978) found that, in 2000 genital tracts that were examined from abattoirs, only six specimens (0.3%) had evidence of congenital abnormalities compared with 194 (9.65%) with acquired lesions. Similar patterns have been demonstrated by Al-Dahash and David (1977a). Anatomical abnormalities usually affect individual cows or heifers and are therefore unlikely to have a major influence on fertility in a herd. Most of the acquired lesions are identified in tracts from parous cows, emphasising the importance of conditions that occur during pregnancy, parturition, and the puerperium. However, heifers also display many similar lesions, some that would usually be associated with pregnancy (Herenda 1987). The results of Al-Dahash and David (1977a) and Herenda (1987) are summarised in Table 22.1.

TABLE 22.1 Incidence of reproductive abnormalities in cattle

	Al-Dahash & David (1977a)	Herenda (1987)
Number of Non-pregnant Tracts	6186 (Mixed Ages)	5800 (15–30 Months Old)
Abnormality	% of Non-pregnant Tracts	
Cystic ovaries		
• Uncomplicated	3.23	12.2
• With corpus luteum	1.52	0.76
• With mucometra	0.22	0.98
Ovarian bursal adhesions	2.39	5.38
Ovarian tumours	0.23	0.06
Hydrosalpinx	1.05	0.22
Salpingitis		0.68
Mucometra	0.23	5.00
Metritis		1.23
Pyometra	1.10	0.18
Non-involuted uterus	2.20	0.07
Segmental aplasia	0.05	0.04
Double cervix		0.26
Freemartin		0.06
Mummified fetus	0.36	0.13
Macerated/emphysematous fetus	0.13	
Uterine adhesions/abscess	0.31	0.06
Segmental aplasia of the vagina		0.44
Fibropapilloma		0.04

From Al-Dahash & David (1977a) and Herenda (1987).

Lesions of the Ovaries

Congenital Lesions

Congenital lesions of the ovaries are rare. A few reports exist of instances in which one or both ovaries are absent (ovarian agenesis), accompanied by an infantile genital tract and an absence of cyclical behaviour. Fincher (1946) saw an apparently hereditary condition of 'virtual absence of ovaries' in three maternal half-sister heifers.

Ovarian Hypoplasia

Ovarian hypoplasia is a little more common than ovarian agenesis. One or both ovaries are small, functionless, and composed of largely undifferentiated parenchyma. Oocytes and follicles are virtually absent. Ovarian hypoplasia is generally a sporadic condition, except in the gonadal hypoplasia syndrome of the Swedish Highland breed.

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A high incidence of gonadal hypoplasia was recognised in males and females of this breed: Lagerlöf (1939) found an incidence of 13.1% of ovarian hypoplasia among 8145 cows. When both ovaries were hypoplastic, the genital tract was infantile, and oestrous cycles did not occur. Eriksson (1943) concluded that the condition was inherited as an autosomal recessive with incomplete penetrance. There was a marked association of gonadal hypoplasia with white coat colour. By the adoption of a vigorous control programme in which veterinary examination of breeding cattle led to the recognition and culling of cases of unilateral hypoplasia, the incidence of gonadal hypoplasia in Swedish Highland cattle was reduced from 17.5% in 1936 to 7.2% in 1952 (Lagerlöf & Boyd 1953). Nonetheless, the disorder is still present in the breed; Settergren (1997) described cases of partial or complete hypoplasia in 18 Swedish Highland heifers.

Ovarian hypoplasia also occurs as an occasional finding in most breeds of cattle, including *Bos indicus* (Abalti et al. 2006). No inherited basis for the condition has been demonstrated in these animals, although, interestingly, Arthur (1959) reported a small number of cases in white Ayrshire heifers, which were acyclical and had hypoplastic ovaries.

Acquired Lesions of the Ovaries

Cystic ovarian disease is the most common of the acquired lesions of the ovaries. It is considered to be a functional disturbance of ovarian function and is described later in this chapter, in 'Cystic Ovarian Disease.'

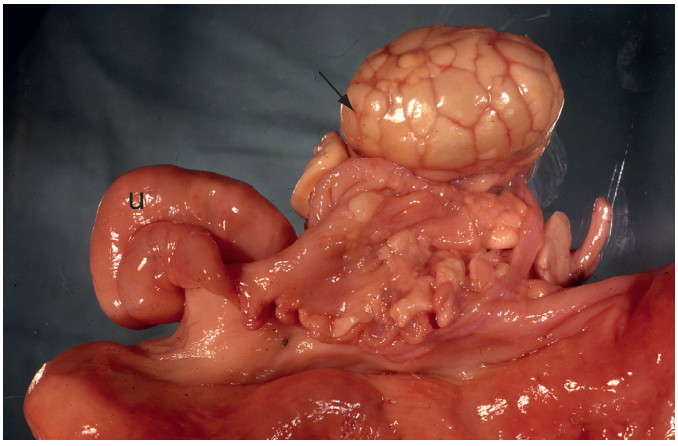
Oophoritis

Ovaritis (oophoritis; see Fig. 22.4) is a very rare lesion of the ovary, usually seen as adventitious findings at postmortem examination. McEntee (1990) described cases of tuberculous oophoritis, brucella-induced oophoritis, and ovarian abscessation in animals that that have had generalised pyaemia. He further suggested that enucleation of the corpus luteum (CL) could be a cause of ovarian abscesses, possibly when it has been undertaken in cows suffering from perimetritis.

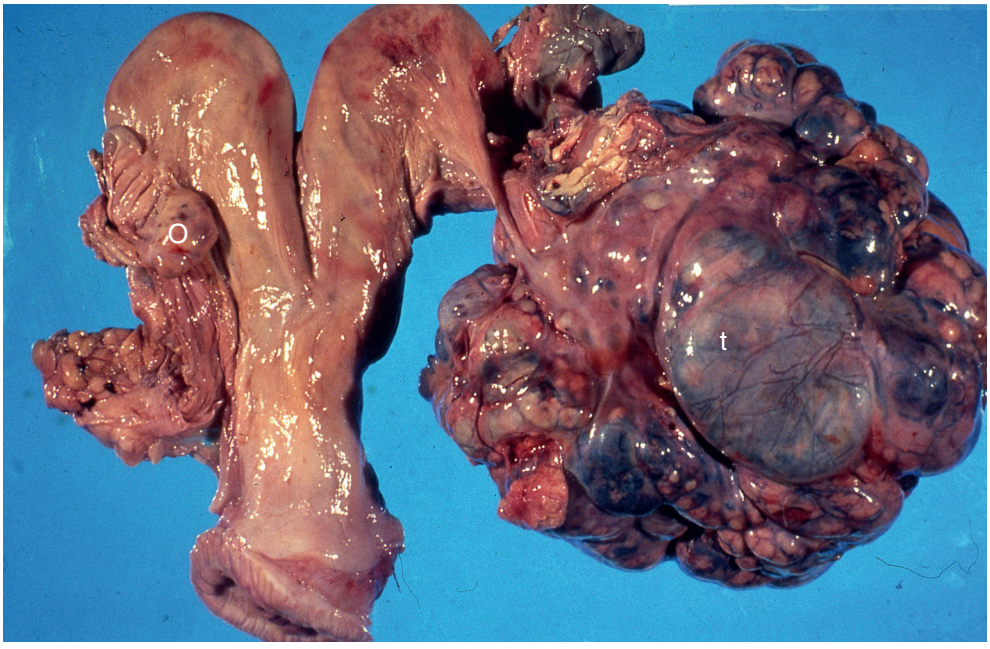
Neoplasia

Neoplasia of the bovine ovary is rare. The tumours most frequently encountered are granulosa cell tumours (Fig. 22.5) and fibromas. Lagerlöf and Boyd (1953) found three granulosa cell tumours and one fibroma in a survey of over 6000 bovine reproductive tracts; Al-Dahash and David (1977a) found seven fibromas and two granulosa cell tumours among 8000 tracts; and Hatipoglu et al. (2002) found three granulosa cell tumours among 1100 tracts. Most granulosa cell tumours are reported from nonpregnant cattle, although the tumour can also occur in pregnant animals. Lagerlöf and Boyd (1953) also found three carcinomas in their survey.

Granulosa cell tumours can produce any of the main ovarian steroids, although reports of oestrogen or androgen production are the most common in the literature (McEntee 1990). Tumours that secrete oestrogens cause animals to display persistent oestrus behaviour, at least in the early stages of the tumour. In long-standing cases, virilism may occur. Progesterone- or androgen-secreting tumours are more commonly associated with anoestrus.



• **Fig. 22.4** Infection and inflammation of the ovary (oophoritis; *arrow*) of an infertile cow. The horn of the uterus is marked 'u'.



• **Fig. 22.5** A granulosa cell tumour (t) involving the right ovary. The left ovary (o) is normal.

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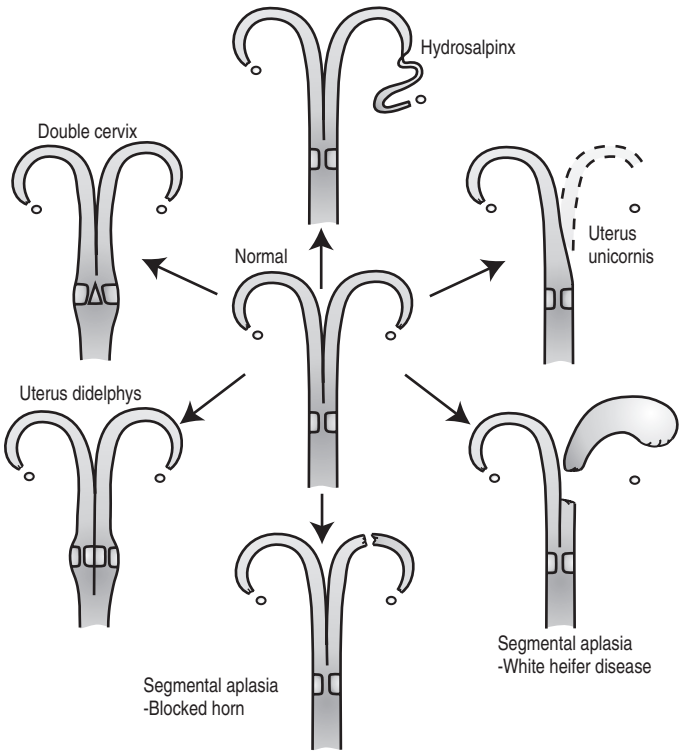
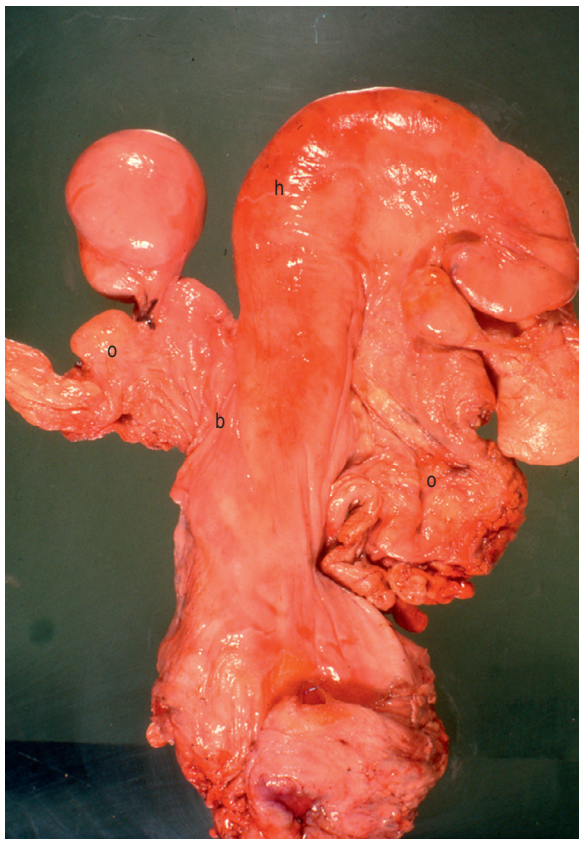


• **Fig. 22.6** Manifestations of the syndrome of uterine segmental aplasia, sometimes known as 'white heifer disease'.



• **Fig. 22.7** Uterus unicornis. Note normal left and right ovaries (o) and complete right horn (h). The left horn comprises a flat band of tissue with no lumen (b) and a blind residual segment.

The unaffected ovary is typically regressed and inactive, although Roberts (1986) reported that conception had occurred in a cow with a unilateral, non-steroidogenic tumour. Granulosa cell tumours are generally regarded as benign, although, in one series, 9 of 13 tumours had metastasised (Norris et al. 1969). McEntee (1990), on the other hand, considered metastasis to be very uncommon. These tumours can become massive: one Friesian cow is reported with a granulosa cell tumour that weighed 24 kg.

Other tumours of the bovine ovary have occasionally been reported. These include carcinomas, fibromas, thecomas, and sarcomas; these tumours are generally benign and are often massive in size. One such cow had an ovarian carcinoma that had enlarged to occupy the caudal third of the abdomen and had metastasised widely throughout the mesenteries.

Abnormalities of the Genitalia

Segmental Aplasia of the Paramesonephric Ducts

Developmental defects of the paramesonephric (Müllerian) ducts lead to a wide range of anomalies of the vagina, cervix, and uterus (Fig. 22.6). Depending on the site of the aplasia, the cow may be subfertile or sterile. However, the ovaries develop normally, and, consequently, affected animals show relatively normal cyclic behaviour. Moreover, because the ovary produces normal levels of steroid hormones, there is a significant level of secretory activity within the tubular parts of the genital tract. Hence when a developmental obstruction of the tubular tract occurs, cyclical secretions can distend the lumen of the isolated portion of the tract.

Aplasia of each part of the tubular genitalia has been reported. In some cases, the whole of the vagina, cervix, and uterine horns may lack patency. In these cases, as in the freemartin, the genital tract is difficult to locate by rectal palpation, but unlike the latter, the ovaries are normal. More commonly, partial or segmental aplasia of the paramesonephric ducts occurs. Youngquist & Braun (1993) identified the uterine tube as the site most often involved; unilateral aplasia was identified in 0.1% of the specimens and segmental aplasia in 0.05%. Herenda (1987) also identified segmental aplasia in 0.04% of 5800 specimens.

In the case of uterus unicornis, only one uterine horn has a lumen with the other appearing as a narrow, flat band (Fig. 22.7); it is more common for the right horn to be absent than the left. Provided the remainder of the genital apparatus is normal, these animals may conceive to ovulations from the intact side. A more serious type of aplasia occurs when isolated sections of uterine horn are present. Uterine secretion accumulates and causes sac-like dilatation of such isolated portions of the tract. These can become very large and can be confused with early pregnancy during manual examination *per rectum* (Figs 22.8 and 22.9). Animals with this deformity are sterile.

Abnormalities of the cervix also occur, usually as a result of fusion of both paramesonephric ducts. In the condition of uterus didelphys (Figs 22.10 and 22.11), failure of fusion is complete, so there is a double cervix, and each uterine horn connects with the vagina by a separate cervical canal. There is often also a division of the cranial portion of the vagina, as this is also derived from the paramesonephric ducts. Such animals may conceive, providing that insemination takes place into the horn ipsilateral to the ovulation; and a number of reports exist of them carrying calves to term and giving birth normally. A more common lesion is partial duplication of the cervical canal, often with a single internal ostium

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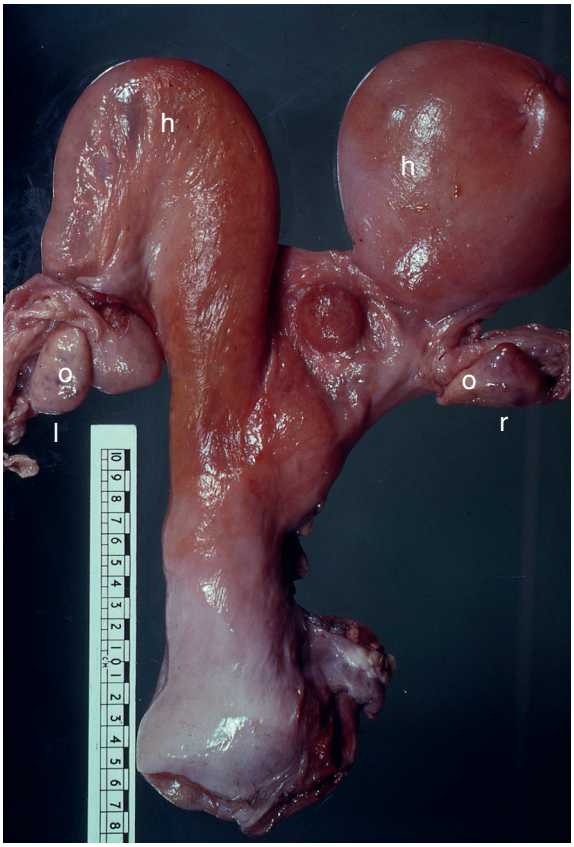
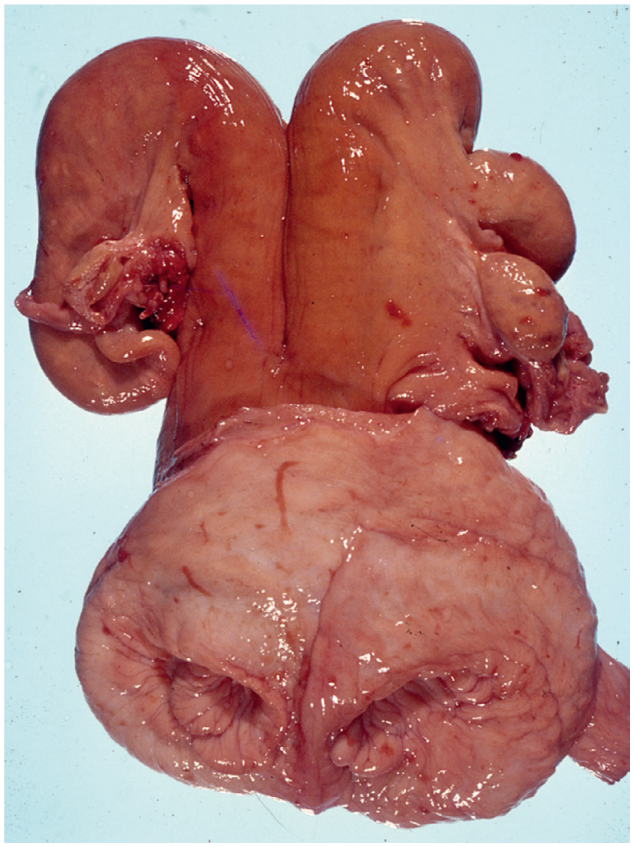
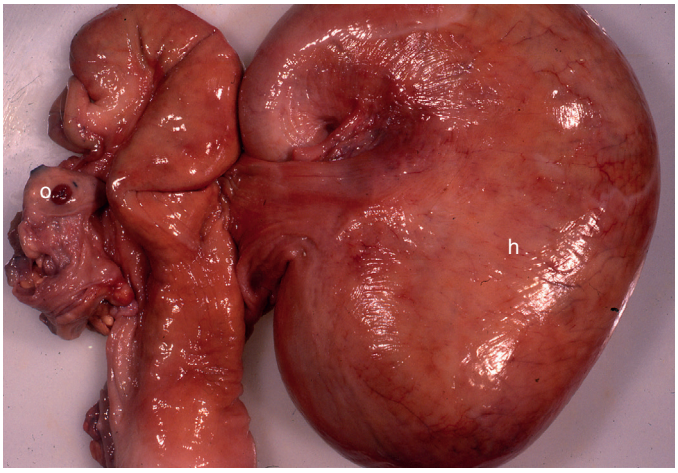


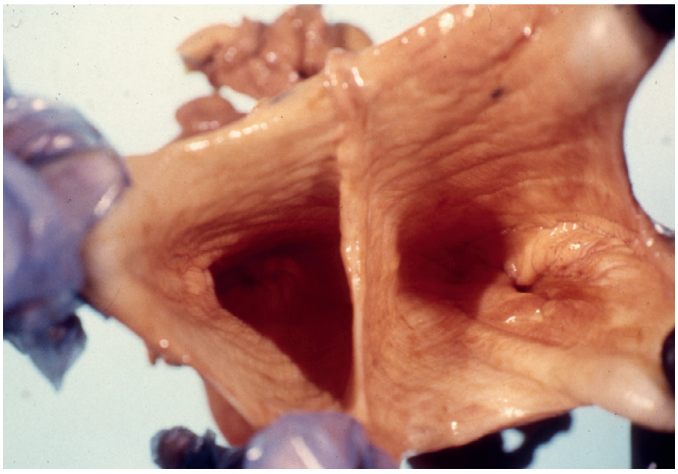
• **Fig. 22.8** Genital tract from a heifer with 'white heifer disease'. Note that both ovaries (o) are normal with a corpus luteum present in the right (r), and the uterine horns (h) are distended with accumulated fluid.



• **Fig. 22.10** Uterus didelphys showing two completely separate cervical canals.



• **Fig. 22.9** Genital tract from a heifer with 'white heifer disease'. Note normal left ovary (o) and isolated portion of the right horn (h) greatly distended with accumulated fluid.



• **Fig. 22.11** Double external os cervix showing two completely separate cervical canals.

and double external ostia. Affected animals conceive normally but may show dystocia due to fetal limbs entering both cervical canals; the expulsion of the afterbirth may also be impeded. A similar complication may arise in cattle showing a dorsoventral post–cervical band.

A further developmental aberration of the female tubular genital tract involves a variable degree of persistence of the hymen. This may appear as a vaginal constriction in front of the urethral opening, as a partition with a central aperture, or as a complete partition between the vulva and vagina. The first type is likely to be discovered at parturition, when it causes dystocia. The second and third types are likely to be found when investigating heifers that either strain forcibly after service or cannot be inseminated artificially. When hymenal obstruction is complete, there is an accumulation of secretions cranial to the obstruction. This may be present on rectal palpation as a fluctuating swelling of variable size or, in more extreme cases, may protrude from the vulva as

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a white, glistening, fluid-filled mass. After service, this retained secretion may become infected by pyogenic organisms. The less severe forms of hymenal obstruction may be rendered suitable for breeding by making cruciform incisions into the partition. Heifers with complete obstruction that are ill because of retained pus can be relieved by trocar and cannula and then fattened for slaughter. However, it should be noted that, in view of the probable hereditary origin of these developmental defects, surgical intervention in order to make breeding possible is not advisable. The genital organs of most heifers with hymenal constriction are otherwise normal, but occasionally segmental aplasia of other parts of the tubular organs or ascending infections of the urinary tract are present.

The aforegoing developmental anomalies may arise in all breeds of cattle. Historically, segmental aplasia was particularly common in the Shorthorn breed, in which it was associated with white coat colour. Hence syndromes associated with segmental aplasia are sometimes referred to as 'white heifer disease'. Originally, 'white heifer disease' was used to describe the syndrome of straining and illness after service in animals with retention of the hymen. Segmental aplasia of the paramesonephric ducts in the Shorthorn is due to a sex-linked recessive gene with linkage to the gene for white coat colour. In other breeds, developmental defects of the paramesonephric ducts are probably also due to either sex-linked or autosomal recessive genes; consequently they are likely to appear when inbreeding is practised. For example, Fincher and Williams (1926) reported that 56% of heifers were affected among the progeny that resulted from the mating of a Friesian sire with his daughters. The disease still occurs; Riley et al. (2007) reported 17 cases of the disease in Holstein–Friesian cattle.

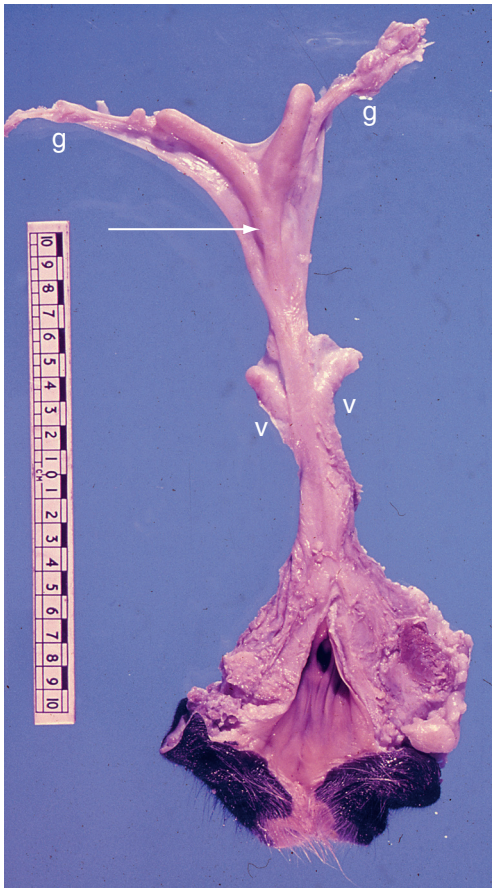
Parovarian Cysts

Parovarian cysts are remnants of the mesonephric ducts that are sometimes present in the mesosalpinx of cows. Tiny parovarian cysts of a few millimetres in diameter are common incidental findings in slaughtered cattle. Larger cysts of between 1 and 3 cm in diameter may be discovered during manual or ultrasound examination of the tract *per rectum* when they may be confused with ovaries. Parovarian cysts are of no consequence to the reproductive performance of the animal except in the rare instances when they impinge on the uterine tube and reduce its lumen.

Freemartinism

Freemartinism (Fig. 22.12) is a distinct form of intersexuality that arises as a result of a vascular anastomosis of the adjacent chorioallantoic sacs of male and female fetuses in multiple pregnancies (see Padula (2005) for review). The majority (~95%) of female fetuses in male/female twin pregnancies are affected (Biggers & McFeely 1966, Zhang et al. 1994). Because vascular anastomosis occurs as early as 30 days of gestation, it is possible for single-born freemartins to occur if there is death of the male twin of a heterozygous pair after the time of vascular fusion with the other being carried to term. This has been demonstrated as a cause of infertility in heifers with apparently normal external genitalia but with sex chromosome chimerism (Wijeratne et al. 1977).

The external genitalia of freemartin heifers may appear relatively normal, although the clitoris is characteristically enlarged and prominent, and coarse hairs are present at the ventral commissure of the vulva (Fig. 22.13). The internal genitalia are grossly abnormal. The gonads are typically vestigial, although they undergo masculinisation in a minority of cases. The structures derived from the paramesonephric ducts are almost entirely absent or are grossly hypoplastic. In animals that have undergone a significant degree of masculinisation, the gonads resemble testes to the extent that their parenchyma contains recognisable tubules and interstitial tissue. Development of the mesonephric (Wolffian) ducts is related to the degree of masculinisation of the gonad. In extreme cases there are well developed epididymides, vasa deferentia, and vesicular glands (Short et al. 1969). More typically, the vestigial gonads of freemartins are devoid of oocytes and follicles and have parenchyma that consists largely of degenerating sex cords. Occasional animals may have some oocytes present in their gonads and may even have small follicles and luteal-like tissue (Rajaoski & Hafez 1963). In some least affected cases, the female genital tract may be small, with a persistent hymen and hypoplastic ovaries (Wijeratne et al. 1977). Presumably the degree of masculinisation/aplasia of the female genitalia can be related to the time at which vascular fusion occurred.



• **Fig. 22.12** Reproductive tract from a freemartin heifer. Note the vestigial gonads (g), underdeveloped structures derived from the paramesonephric ducts (arrow) and rudimentary vesicular glands (v).

The newborn freemartin can sometimes be recognised by its prominent clitoris and tuft of hair at the ventral commissure of the vulva, although these signs are not always reliable. Freemartins can be identified on the basis of the length of the vagina and the absence of the cervix. In the adult the vagina is normally 30 cm in length compared with 8 to 10 cm in the freemartin. Rectal palpation will fail to identify the cervix. In calves of 1 to 4 weeks of age the vagina is normally 13 to 15 cm in length compared with 5 to 6 cm in a freemartin. Diagnosis at this age can be made using a blunt probe, which should be inserted initially at an angle of 45 degrees below the horizontal for 5 cm and then angled downwards

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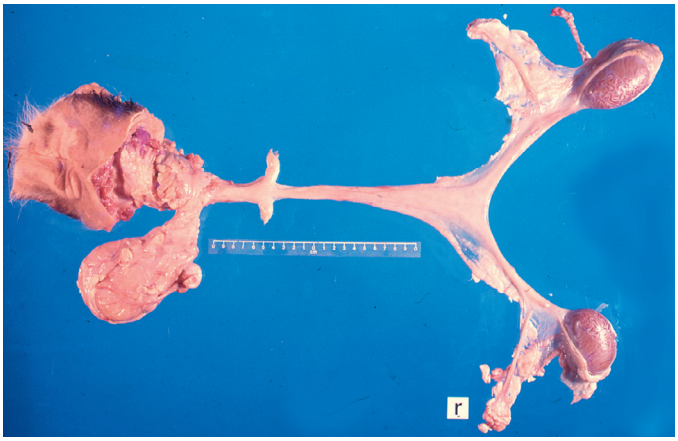
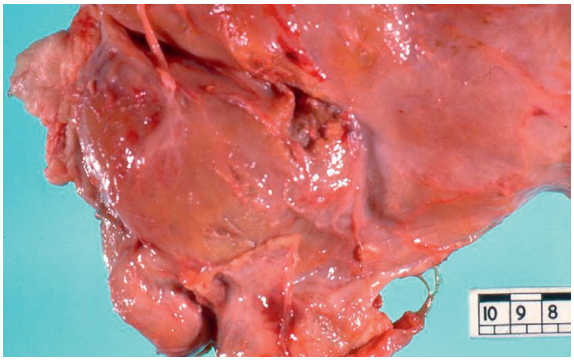
• **Fig. 22.13** Vulva of a freemartin heifer showing the prominent clitoris and coarse hairs at the ventral commissure.



• **Fig. 22.14** Sex reversal in a Hereford cow. The vulva and vestibule have the phenotypic appearance of a normal female, but the structures derived from the paramesonephric ducts are absent. The gonads resemble testes, and epididymis-like structures are present. The mesonephric ducts are largely undeveloped, although vestigial vesicular glands are present.



• **Fig. 22.15** Ovariobursal adhesions. Note that the ovarian bursa has completely enveloped the ovary.

to avoid impinging on the hymen (Long 1990). It is easier when comparisons can be made among a number of animals.

The most accurate method of diagnosis, although not absolute, is the demonstration of sex chromosome chimerism in cultured lymphocytes. Heifer calves that are born cotwins to males and show morphological changes in their reproductive tracts invariably show sex chromosome chimerism in blood and blood-forming tissues. Unfortunately, the distribution of male cell percentages in freemartins appears to be random; thus animals with low male percentages in the blood are as common as those with high male percentages (Wilkes et al. 1981). More recently, diagnosis by a polymerase chain reaction (PCR) test for the presence of Y chromosomes in blood cells or hair has been described (Plante et al. 1992). This is a definitive test for the presence of the Y chromosome but does not provide an absolute differentiation between freemartinism and other abnormalities of sex differentiation in which a Y chromosome is present.

The economic importance of early diagnosis of freemartinism has been shown by the survey of David et al. (1976), who found that a large number of heifers that were sold for breeding in markets were freemartins. Very high incidences of freemartinism can sometimes be found therefore in herds of heifers – most commonly those of heifer rearers – that are purchased from markets. This could also become important if induction of twinning by superovulation or embryo transfer becomes popular. Increased emphasis on animals being sold on the basis of being 'fit for purpose' is putting pressure on farmers to declare if a heifer was born cotwin to a bull to avoid subsequent litigation.

Very occasionally, other forms of intersexuality are found. A few cases of pseudohermaphroditism have been reported, as have rare cases of XY sex reversal (Fig. 22.14) and true hermaphroditism.

Atresia of the Vulva

An abnormally small vulva has been described as a cause of dystocia in Friesian and Jersey heifers. In such cases episiotomy or Caesarean operation may be required to allow delivery. The defect has been seen to affect many of the progeny of a particular Jersey bull (Hull et al. 1940), indicating that it is likely to be of hereditary origin. It is occasionally seen in newborn calves in conjunction with atresia ani (Wamaitha et al. 2015).

Ovarian Bursal Adhesions

Acquired lesions of the uterine tubes and adnexa are common in cattle. One of the most frequently observed lesions of the bovine reproductive tract is adhesion between the ovary and the ovarian bursa (Fig. 22.15). The incidence of ovarian bursal adhesions ranges from 0.43% (Summers 1974; Australia), 0.97% (Lawton et al. 2000; New Zealand), 1.83% (Al-Dahash & David 1977a; UK), to 5.38% (Herenda 1987; Canada). The condition is uncommon in heifers, but its incidence increases with the age of the cow.

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Much variation exists in the extent of the adhesions that are present, ranging from fine, web-like strands in the depth of the bursa, which do not involve the uterine tube, to intermediate cases with fibrinous or fibrous strands of varying thicknesses connecting the fimbriae or bursae to the ovary, to complete envelopment of the ovary within a closely applied fibrous bursa. Infections of the ovarian bursa also occur, which are invariably accompanied by large-scale adhesions between the ovary and bursa, often in association with metritis or salpingitis. Ovarian bursal adhesions are also associated with cystic ovarian disease, although it is not clear which lesion is causal. In such animals regressed luteinised follicles from past cycles may be present in the same ovary. Strand-like adhesions arising from scars of old CLs (which more commonly affect the right ovary) may be regarded as physiological hazards that originate as slight haemorrhages from the site of ovulation. It is possible that a proportion of ovarian bursal adhesions occur as a result of rough palpation of the ovaries, particularly if a CL is accidentally enucleated or an ovarian cyst is burst. Other causes of ovarian bursal adhesions include salpingitis, metritis, or local or diffuse peritonitis (including tuberculous peritonitis). Indeed, the most likely cause of ovarian bursal adhesions in the pluriparous cow is puerperal infection, which arises from ascending infection of the uterus.

Minor, web-like adhesions are unlikely to interfere with fertility but, of the remaining more severe types, between 25% and 50% are bilateral and likely to interfere with ovulation or to impede sperm or egg transport through the uterine tube. Of the unilateral cases, the right side is more frequently involved. Conception is unlikely to occur to ovulations from the affected side. When the bursa is diffusely adherent to the ovary, ovulation is prevented and luteinisation of the follicle occurs, with the orange rim of the follicle being several millimetres thick. Intraovarian and periovarian abscesses have also been seen (Arthur & Bee 1996) in association with ovarian bursal adhesions.

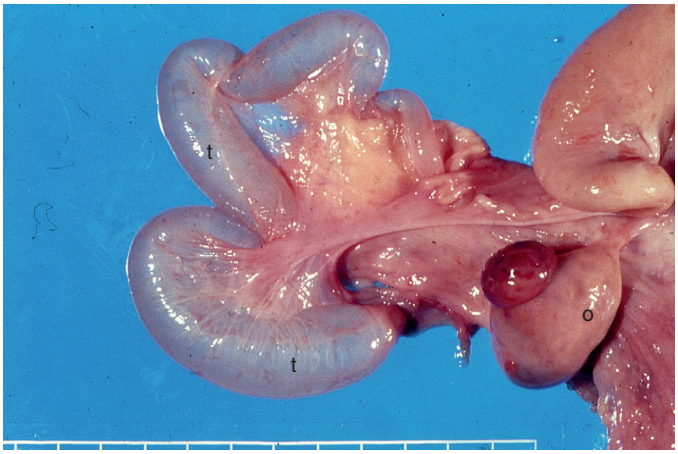
There is no doubt that ovariobursal disease is one of the major causes of individual cow infertility characterised by regular return to oestrus. There is no satisfactory treatment for the condition, although prompt attention to cases of dystocia with a view to preventing puerperal metritis would also reduce the incidence.

Lesions of the Uterine Tube

Occlusion of the lumen of the uterine tube results in an accumulation of secretions, resulting in thinning and distension of the wall, which is described as hydrosalpinx (Fig. 22.16). The accumulation of fluid is often a result of adhesions occluding the lumen, but it may also occur as the result of congenital stenosis or infection. Gross infection of the uterine tube, known as pyosalpinx, may occur as a result of a super infection of a hydrosalpinx with *T. pyogenes* or may be the result of ascending infection from the uterus. Most of such cases are the result of severe metritis or perimetritis, although cases have been reported in association with the presence of pyometra or of a macerated fetus (Kessy 1978). Pyosalpinx can often progress to pyobursitis. Because infection can be forced up the uterine tubes during irrigation of the uterus (e.g., in an animal with metritis), care should be exercised during this procedure.

Salpingitis and ovarian bursal adhesions can also be caused by *Mycoplasma* and *Ureaplasma* infections (Yaeger & Holler 2007). Ovariobursal adhesions are a feature of the viral epididymovaginitis of cattle in east Africa.

Several other acquired abnormalities of the uterine tubes can also cause infertility. A condition described as pachysalpinx has been identified in three genital tracts from parous animals (Kessy 1978). The gross appearance of the tube resembles hydrosalpinx or pyosalpinx, but no fluid is contained within the lumen; instead there is a mass of connective tissue. Enlargement and distension of the uterine tube can also occur as a result of the presence of multilocular mucosal cysts containing PAS-staining gelatinous material.



• **Fig. 22.16** Hydrosalpinx. Note distended ampulla of the uterine tube (t). The ovary (o) is unaffected and contains a corpus luteum.

Diagnosis of Ovarian Bursal Disease and Occlusion of the Uterine Tubes

Diagnosis of ovarian bursal adhesions in the live animal is difficult. As a consequence, only about one-third to one-half of the lesions that cause infertility are diagnosed by rectal palpation. In the more severe cases of ovarian bursal adhesions, the periphery of the ovary loses its clear definition, the ovarian outline is more bulky and irregular, and the ovarian mass lacks mobility. In occasional difficult cases, laparotomy with direct vision or endoscopic examination of the ovaries may be used. Rectal palpation of distended uterine tubes is usually indicative of the presence of advanced lesions of hydrosalpinx or pyosalpinx; ultrasonography has also been used to diagnose more advanced lesions (Pereira et al. 2015). However, most cases of occlusion of the uterine tubes can only be determined by undertaking tests of their function.

A fairly simple test is available to assess the patency of the uterine tube. Speck (1948) first demonstrated that if phenolsulphonphthalein (PSP) was placed in the uterine lumen, it was not absorbed, but if the uterine tubes were patent, it passed along them into the peritoneal cavity from where it was readily absorbed into the circulation. The PSP was then excreted by the kidneys into the urine, where it produced a red or pink colour if alkaline. If the uterine tubes were occluded**,** there was no passage of dye and hence no discoloration of the urine. The test involves the infusion of 20 mL of a 0.1% sterile solution of PSP into the uterine lumen, using a Nielson catheter. This has to be done carefully so as to avoid any trauma to the endometrium enabling absorption to occur. The bladder should then be catheterised, a small sample of urine is kept for a control, and a further urine sample is then collected 30 to 60 minutes later. The urine is made alkaline by the addition of 0.2 mL of 10% trisodium orthophosphate buffer to 10 mL of urine. In the presence of PSP, the liquid becomes red or pink; in its absence the urine remains the same colour as the control. The test should be performed during the luteal phase of the cycle, preferably about Day 10, because false negatives can be obtained during the follicular phase. False positives can arise if

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• **Fig. 22.17** Mucometra as a consequence of long-standing, untreated cystic ovarian diseases. Note the thin wall of the uterus and the presence of an ovarian cyst.

there is endometrial erosion due to infection and inflammation; it is not very effective in differentiating between bilateral and unilateral patency (Kessy & Noakes 1979a, b).

A more accurate method of evaluating the patency of each uterine tube separately has been described by Smith (2004). A Foley catheter is introduced into one horn, the cuff is inflated, and a small volume of dye is infused into the tip of the horn. If the tube is patent, the dye will pass via that uterine tube to the peritoneal cavity and hence to the urine (the cuff prevents reflux of the dye to the other side). The procedure is repeated on the other side several days later.

The prognosis for the condition is, at best, guarded. In cases of bilateral occlusion of the uterine tube the animal is normally irreversibly sterile. Various methods of restoring patency to the uterine tube have been described, but they appear to be of negligible therapeutic value.

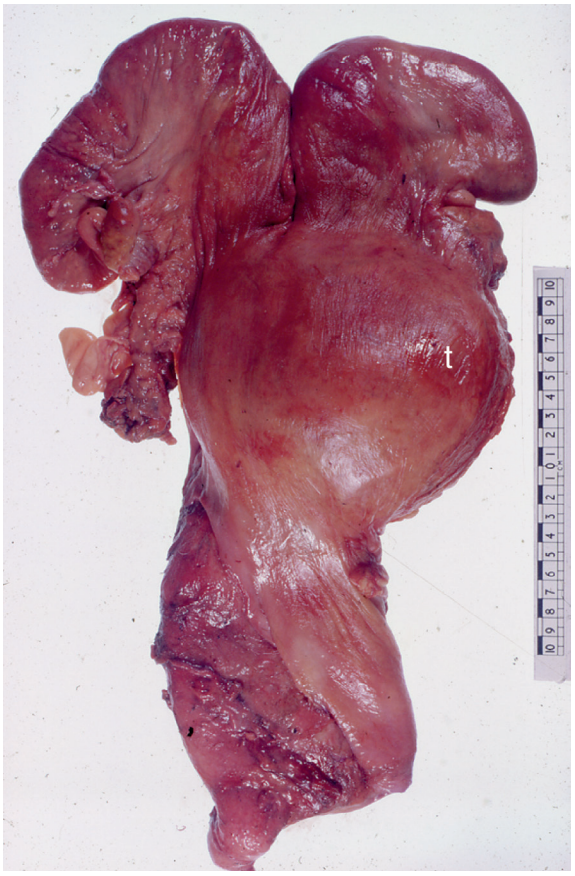
Mucometra

Mucometra is a rare sequel of untreated cystic ovarian disease. In this condition the uterus fills with a mucoid fluid, accompanied by a marked thinning of its wall and degeneration of the endometrium (Fig. 22.17). The enlargement of the uterus is bilateral, but is typically asymmetrical, so care is needed to differentiate the condition from a Week 9 to 11 pregnancy on rectal palpation. The ultrasonographic appearance should be easy to differentiate from pregnancy. Affected cows are most unlikely to be able to conceive.

Uterine Tumours

Tumours of the uterus are rare in cattle. Of those that do occur, benign tumours of mesenchymal tissues are the most common. Leiomyomas and fibromyomas have been described in cattle from many countries, and there have been significant numbers of reports of lymphosarcomas of the uterus of cattle in North America. In a meta-analysis of abattoir surveys of reproductive abnormalities, Roberts (1986) found that leiomyomas, fibromyomas, and fibromas accounted for 77% of tumours. These benign tumours are often incidental findings at the time of slaughter, although they affect fertility if they occlude or occupy a significant proportion of the uterine lumen. Very occasionally, the presence of a tumour may be found in a pregnant uterus. Such tumours are often large (Fig. 22.18) and can potentially be mistaken for the presence of a mummified fetus on palpation *per rectum*. Occasionally, the tumours are massive.

However, other series of cases have reported adenocarcinoma and lymphosarcomas to be the most important tumours of the bovine



• **Fig. 22.18** Fibroma (t) involving the base of the uterine horns and body.

uterus (Brandley & Migaki 1963, Smith 1965). Adenocarcinomas present as moderately enlarged, firm, constricted lesions of the uterine wall (McEntee 1990) and have a high rate of metastases to the lung and abdominal structures. Affected animals often present clinically as having chronic wasting disease. These tumours are very rare in Europe; most cases have been reported from North America.

Uterine Adhesions

Uterine adhesions are a common sequel of perimetritis. Adhesions may be present as fibrinous tags over the surface of the uterus (Fig. 22.19), adhesions that involve the ovarian bursa, or adhesions to other pelvic/abdominal viscera. A similar lesion may follow uterine rupture or retention of the fetus after dystocia. A troublesome sequel to Caesarean hysterotomy is adhesion of the uterus to the omentum, intestines, or abdominal wall, or ovarian bursal adhesions that occur when infected uterine fluids contaminate the bursa during surgery. Such lesions are frequently associated with sterility.

Lesions of the Cervix

The role of the cervix as the main barrier between the uterine lumen and the external environment makes it vulnerable to both infections and to trauma. Inflammation of the cervix is likely to follow obstetrical trauma incurred during the relief of a difficult dystocia. The most common congenital lesions of the cervix are those that occur as part of the 'white heifer' syndrome.

Cervicitis almost invariably accompanies puerperal metritis, and is common in cases of delayed involution of the uterus and/or retention of the afterbirth. The organisms present in such infections are those normally found in the caudal vagina, including *Escherichia*

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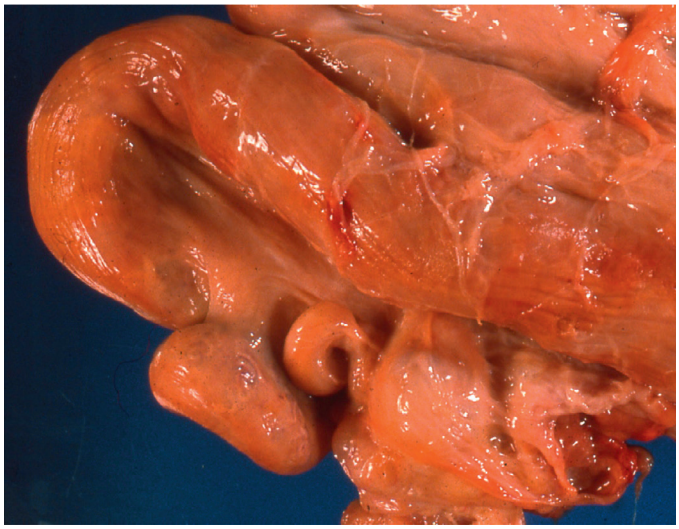
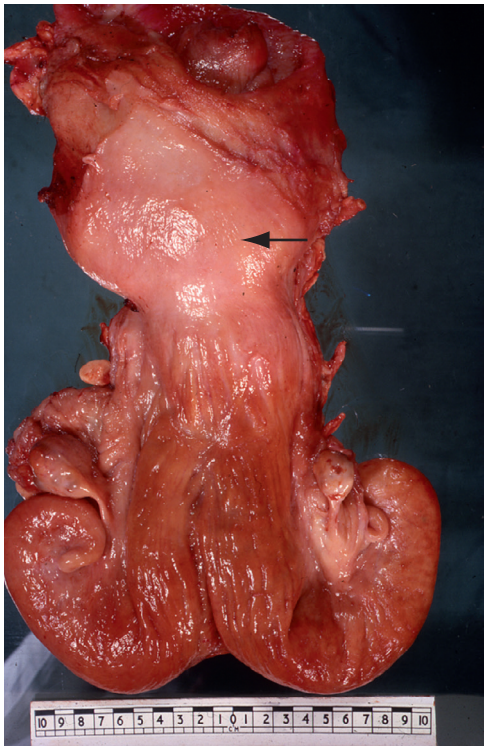


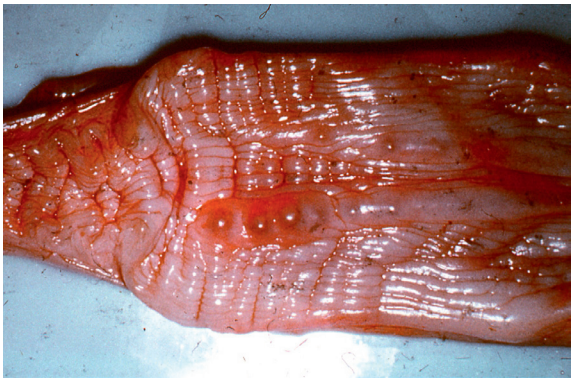
• **Fig. 22.19** Uterine adhesions present as fibrinous tags over the surface of the uterus, probably as a result of perimetritis. Note the adhesions of the ovarian bursa. (Courtesy Massey University Press. In: Parkinson, et al. *Diseases of Cattle in Australasia,* 2010.)



• **Fig. 22.20** Fibroma of the cervix (the cervix is *arrowed*).

coli, streptococci, staphylococci, and *T. pyogenes.* The latter organism is most prominent in established infections. Rarely, parturient laceration of the cervix is followed by fibrosis and obstruction of the cervical canal, resulting in infertility. Fibrosis of the cervix may very occasionally prevent its normal dilatation at the next parturition. Although prolapse of one or more of the cervical folds is commonly seen in the plurigravid cow, it is a hazard of parturition rather than a cause of infertility.

Tumours of the cervix (Fig. 22.20) occur very occasionally. Leiomyomas and, to a lesser extent, fibromas are the most common of these lesions, whereas adenocarcinomas (the most common human



• **Fig. 22.21** Cystic dilation of Gartner's glands.

cervical tumour) are very rare indeed. The benign tumours of the cervix are only of clinical significance as space-occupying lesions or when they cause mechanical interference.

Mummified and Macerated Fetus

These conditions are described in Chapter 9.

Conditions of the Vagina, Vestibule, and Vulva

Cysts of Gartner's Canals

Cysts in linear series, which may be 6 to 8 cm in diameter, often occur on the floor of the vagina (Fig. 22.21). They can be easily punctured and are not a cause of infertility.

Obstetrical Damage to the Vagina

Parturient trauma of the tubular genital tract is a frequent consequence of dystocia. Fetomaternal disproportion is the most common cause of dystocia in cattle, particularly in the Holstein–Friesian breed, and, as a consequence, delivery of large calves by forced traction can damage the birth canal to such an extent that the animal is rendered sterile. Obstetrical contusion of the vagina, especially in fat heifers of the beef breeds, is particularly likely to be followed by necrotic vaginitis associated with *Fusobacterium necrophorum* infection. Likewise, in other instances involving the removal of dead, emphysematous calves in unhygienic circumstances, parturient trauma may be followed by severe toxaemia due to invasion by anaerobic bacteria. Treatment of such animals requires the use of parenteral broad spectrum antibiotics and, in severe cases, supportive fluid therapy. Some practitioners have found that local emollient creams are also helpful. Because vaginitis causes persistent straining, particularly if severe, caudal epidural anaesthesia will provide temporary relief.

Other sequelae of dystocia include laceration or bruising of the vulva, which may be followed by cicatrisation and distortion, with imperfect closure of the vulval sphincter and aspiration of air. These sequelae are similar to, but less severe than, those of rupture of the perineum. Some of these cows are infertile to natural service but conceive to intrauterine insemination. At subsequent parturition, dystocia owing to fibrosis of the vulva may arise. Gross fibrosis of the vagina may also follow pyogenic infection of lacerations, causing a narrowing of the birth canal and dystocia; Caesarean surgery may then be required at subsequent births.

Obstetrical Damage to the Perineum

Lacerations of the perineum can also result in impaired fertility of affected cows. Second degree perineal lacerations may give rise to a

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pneumovagina; if the conformation of the vulva is compromised, surgical correction is possible by performing Caslick's operation.

A third degree perineal rupture may occur at calving, usually as a result of dystocia and severe calving trauma. In this situation the whole thickness of the vagina and rectal wall ruptures so that the rectum and vagina are confluent (i.e., the cow has a cloaca). Third degree perineal tears do not heal, thus air and faeces are aspirated into the vagina, inevitably resulting in vaginitis, cervicitis, and metritis. Affected cows have a chronic mucopurulent vulval discharge, but their general health is not impaired; normal cyclic behaviour resumes, but conception does not occur because of the metritis (see later in this chapter). The condition can be cured only by surgical reconstruction of the perineum. Rupture of the perineum may be prevented by sound obstetrical technique, including episiotomy.

These conditions are discussed in detail in Chapter 20.

Urovagina

An increasing number of cows are being diagnosed as having vaginal urine pooling or urovagina. In such animals, urine accumulates in the anterior vagina, where it impinges upon the cervix and causes inflammation of both cervix and vagina; the inflammation then extends into the uterus, causing endometritis. There seems to be a greater prevalence in certain breeds, particularly Charolais and Holsteins. The cause of the condition is not known, although stretching of the suspensory apparatus of the genital tract as a result of several pregnancies may be a factor. Surgical treatment has been described (Hudson 1986), although the procedure is far from straightforward.

Tumours of the Vagina and Vulva

Fibropapillomas of the vagina and vulva of cattle are not uncommon. They do not cause infertility but may interfere with birth. They are usually pedunculated and may be removed surgically. It is likely that there is one form of vaginal fibropappiloma which is of viral origin and is transmitted venereally; it occurs in young cattle and undergoes spontaneous resolution.

All other tumours of the vagina and vulva are rare. A squamous cell carcinoma of the vulva occurs in unpigmented areas of the skin of cattle that are exposed to high levels of solar radiation (McEntee 1990). Lymphosarcomas have also been occasionally found in the vagina.

Functional Infertility

Anoestrus

Anoestrus may be due to:

- Pregnancy;
- Anovulatory anoestrus;
- Cystic ovarian disease, which can result in anoestrus, or other abnormal patterns of reproductive behaviour; and
- Prolongation of the life span of the corpus luteum. This most commonly occurs as a consequence of endometritis, but can occur spontaneously (Kafi et al. 2012) as a result of embryonic death after the maternal recognition of pregnancy.

Pregnancy

It is remarkable how often pregnant cows, many in advanced gestation, are presented for examination for the cause of anoestrus. Most commonly, this occurs when dates of artificial inseminations or natural service matings are not recorded; but accidental situations, such as the bull that breaks through a fence or animals that have not been castrated, are by no means rare. Hence whenever anoestrus cows are examined, the possibility of pregnancy should not be overlooked however adamantly the herd manager protests the impossibility of its occurrence.

Anovulatory Anoestrus

Oestrous cycles, which cease during pregnancy, do not resume immediately after calving. The high concentrations of progesterone that have been present during pregnancy cause negative feedback suppression of the hypothalamo–pituitary axis, with the result that follicular activity in the ovaries of full term pregnant cows is minimal. A period of restoration of both gonadotrophin secretion and ovarian follicular activity occurs during the postpartum period before oestrous cycles are resumed, and a period of postpartum anovulatory anoestrus is a normal aspect of bovine reproduction (see Chapter 7). However, anoestrus becomes a problem when it extends into the period when animals need to be rebred. When this occurs, it is considered to be pathological and needs treatment. At the herd level, it is both the duration of anoestrus in each individual cow and its prevalence within the herd that determine the significance of the problem for the maintenance of regular calving patterns.

The clinical syndrome of anoestrus most frequently manifests as a prolonged period of postpartum anoestrus. Some other cows resume oestrous cycles at the normal time after calving but later relapse back into anoestrus. Other cows will start to have cycles, be inseminated, fail to conceive, and thereafter relapse into anoestrus. These animals are associated with significant economic losses, as their failure to return to oestrus may well be regarded as a sign that they have successfully conceived (Cavalieri & Macmillan 2002).

Prevalence

True anoestrus is most frequently diagnosed in high yielding dairy cows, first-calf heifers that are still growing, and beef suckler cows. Observed frequencies of anovulatory anoestrus are dependent on the presence or absence of the factors that predispose to the development of the condition. Thus a generalised figure for prevalence is of limited value because it depends entirely upon both the type of cattle and the management system. Even within a single system, the occurrence of anoestrus varies from region to region, season to season, and, especially from year to year. Interactions between nutrient availability, climatic conditions, and the establishment of lactation are responsible for enormous fluctuations in the incidence and depth of anoestrus.

Pathogenesis

Anovulatory anoestrus is a multifactorial problem, which occurs in response to a range of management or nutritional deficiencies. Major predisposing factors include:

Breed. The time taken for reestablishment of oestrous cycles after calving is generally longer in beef breeds (36–70 days) than in dairy breeds (10–45 days). The delayed resumption of oestrous cycles in beef cows is due to the combined effects of suckling and nutritional status (Sinclair 2008).

Season. The season undoubtedly affects the prevalence of anoestrus, although its effects vary by geographical location, depending on the interaction between climate, day length, state of nutrition, and the characteristics of the breed being studied. For example, in the temperate climate of Flanders, Opsomer et al. (2000) reported the lowest prevalence of anoestrus in high yielding dairy cows that calved in September (autumn) and the highest in April (spring),

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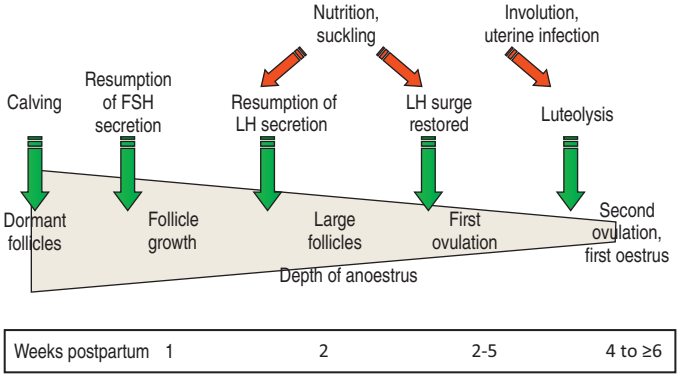
whereas in Iraq, the proportion of Holstein cows that were anoestrous was higher in winter than in autumn (Omran & Al-Saffa 2011).

Nutrition. Imbalances between the energy requirement for lactation and the energy available in feed are probably the main cause of anoestrus. Protein is less commonly limiting, unless gross deficiencies occur. Energy-related metabolic disorders, such as fat cow syndrome, fatty liver, and ketosis, are also related to anovulatory anoestrus. Very high metabolic workload, such as that which occurs in high yielding dairy cows, is also associated with anoestrus, even when the energy and protein requirements of the cow are fully met (Muller et al. 1986, Rind & Phillips 1998). Trace element deficiencies (notably magnesium, phosphorus, copper, cobalt, and manganese) are commonly associated with anoestrus. Sometimes these are causal; others may be reflective of, or additive with, an inadequate supply of energy (McClure 1994).

Stress. Social stresses produced by group and space management of animals are widely considered to be a significant contributor to anoestrus (Platen et al. 1995, Albright & Arave 1997). Physical stressors such as transport (Nanda & Dobson 1990), temperature, and handling (Thun et al. 1996), have also been implicated as limiting factors to reproduction in cattle, as have the stresses of pain and intercurrent disease.

Lameness. Lameness predisposes cows to anoestrus because of its effects upon nutrition and via the stress produced by chronic or unrelieved pain. Lame cows have longer intervals from calving to first service and from calving to conception, and have lower conception rates (Lucey et al. 1986), lower submission rates, and lower final pregnancy rates (Morton 2000a). Lame cows usually have subnormal feed intakes, so they often lose weight rapidly. Hence when lameness occurs during early lactation, affected cows are in negative energy balance for a long period of time. It is likely that there are also corticosteroid-mediated effects on the reproductive endocrine axis (Ley et al. 1994).

The endocrine mechanisms which are involved in restoration of normal cyclic activity of the reproductive system after calving are summarised in Fig. 22.22 (and see Chapter 7). The process is initiated by the hypothalamus regaining the ability to produce GnRH, as the negative feedback effects of the high progesterone and oestrogen concentrations in late gestation are withdrawn. There is a rapid increase in follicle-stimulating hormone (FSH) concentrations between approximately 7 and 14 days postpartum, resulting in the emergence of the first follicular wave. Whether the first dominant follicle ovulates depends on the growth and development of the follicle and the ability of the pituitary to sustain both an adequate luteinising hormone (LH) pulse frequency for preovulatory



• **Fig. 22.22** Sequence of event during the restoration of the reproductive endocrine axis during the postpartum period.

growth and an LH surge to cause ovulation (Roche 2006). The postpartum elevation of FSH secretion is largely independent of nutritional factors, so the extent to which follicular growth is adequate is largely determined by the systemic concentrations of the metabolic hormones, insulin, and insulin-like growth factor (IGF)-I (Butler & Smith 1989, Lucy 2001). Hence situations in which low IGF-I concentrations are present (underfeeding, Holstein genetics) impede follicular growth and reduce the chances of ovulation of the dominant follicle (Roche 2006).

Restoration of pulsatile LH secretion and the LH surge mechanism takes rather longer than that of FSH. In the early postpartum period, the anterior pituitary is virtually refractory to stimulation with GnRH, with progressive increases in responsiveness taking place over the period between approximately 14 and 28 days after calving (Lamming et al. 1979). However, negative energy balance reduces LH pulsatility (Beam & Butler 1999, Butler 2000) through effects of central hypoglycaemia (McClure 1994, Funston et al. 1995), excess concentrations of products of tissue lipid mobilisation (Zaaijer & Noordhuizen 2001), low circulating insulin concentrations (Grummer et al. 2004), and low concentrations of IGF-I (Lucy 2001). If the LH pulse frequency is adequate, the increase in oestradiol concentrations necessary to induce positive feedback and ovulation will occur. If not, the dominant follicle will undergo atresia, and a new follicular wave will be initiated. Hence the interval between calving and the resumption of normal pulsatile LH secretion is a key determinant to regaining the ability to ovulate (Lamming et al. 1981) and the resumption of oestrous cycles.

In beef cows, suckling contributes to the duration of anoestrus. There is much evidence that this is mediated by a reduction in the secretion of LH and consequent failure of follicles to ovulate (Sinclair et al. 2002). It was originally thought that this suppression of LH secretion in suckled animals was due to the raised concentrations of prolactin that are found in animals that are being suckled (Karg & Schams 1974), and there is evidence for such an effect in the effects of dopamine (= prolactin inhibiting factor) agonists and antagonists upon LH secretion (Peters & Lamming 1990). However, prolactin concentrations are not particularly well correlated with the resumption of cyclical activity (Wheeler et al. 1982), so other factors, such as endorphins/opioids, have been suggested as an alternative mechanism (Yavas & Walton 2000). Sinclair (2008) suggested that the inhibitory effects of suckling are mediated through an increased sensitivity of GnRH secretion to oestradiol negative feedback which, in turn, is mediated through central effects endorphins/opioids and insulin upon the hypothalamo–pituitary axis. Thus there appears to be a unifying mechanism for the effects of poor body condition, low plane of nutrition, and suckling in terms of the central effects of insulin upon the reproductive endocrine axis.

Stress contributes to the development of anovulatory anoestrus primarily through its effects upon gonadotrophin secretion. The effects of stress are initially mediated primarily through corticosteroid-mediated pathways (Smith & Dobson 2002), acting through Type II glucocorticoid receptors (Breen et al. 2004). Not all the effects of stress are corticosteroid-mediated, inasmuch as the impairment of reproductive function that is induced by stress persists after adaption and reduction of corticosteroid concentrations towards resting values (Smith & Dobson 2002). Corticosteroid-mediated pathways inhibit LH pulse frequency via a reduction in GnRH pulsatility (Smith et al. 2003) and a reduction in the response of the pituitary to GnRH (Breen et al. 2005). Consequently, there is impaired preovulatory follicular growth and oestradiol synthesis,

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and a delay or block in the development of the LH surge. There are also a number of corticosteroid-independent mechanisms that inhibit LH pulsatility, including opioids, corticotrophin-releasing hormone (Smith et al. 2003), endotoxin-mediated (Debus et al. 2002), and other mechanisms that potentiate the negative effects of hypoglycaemia upon the central neuroendocrine axis.

Clinical Findings

The clinical history is normally either of an animal that: (1) has not been seen in oestrus since the time of calving; (2) started having oestrous cycles but has subsequently ceased; or (3) is presented for pregnancy diagnosis but is found to be non-pregnant. The latter cows may have relapsed into anoestrus, but some will have a persistent corpus luteum or endometritis. Cuttance and Mason (2015) found an equal proportion of such cows with inactive ovaries or persistent corpora lutea.

On examination *per rectum,* the ovaries of the majority of affected cows are small, quiescent, flat, and smooth. This is especially so in first-calved heifers. A small proportion of anoestrous cows have larger follicles (up to a prematuration size of 1.5 cm) present. Depth of anoestrus can be estimated by the size of the ovaries and the degree of development of the structures within them (Nation et al. 1998). Thus cows with very small, inactive ovaries that are devoid of any significant structures (i.e., no palpable follicles or luteal structures) are likely to be in a greater depth of anoestrus than those with larger ovaries containing palpable follicles.

Differentiation must be made from other causes of anoestrus and from ovaries that are functioning normally but are at a phase of the oestrous cycle that does not have prominent ovarian structures. The presence of a mature corpus luteum is generally associated with pregnancy, endometritis, or pyometra, whereas the presence of cystic ovarian disease is also characterised by enlargement of the ovary. Old cows frequently have roughened, irregular ovaries because of the presence of old corpora albicantia. Corpora lutea are difficult to palpate or to visualise by ultrasonography in the early postovulatory period, and the ovary of the freshly ovulated cow can be difficult to differentiate from an inactive ovary (although the presence of uterine tone and vaginal mucus should make the differentiation clear). Milk or blood progesterone can help to determine whether active luteal structures are present, with the development of 'cowside' ELISA tests giving the potential for this to be done when the cow is being examined (Bajema et al. 1994).

Management

In the long term, a high incidence of anovulatory anoestrus in a herd is best managed by identification and correction of the predisposing factors. For example, feeding could be improved, micronutrient deficiencies corrected, and stress reduced. In the short term, affected animals can be treated with reproductive hormones in an attempt to 'restart' the reproductive endocrine system. Even though such hormonal treatments are to a greater or lesser extent successful, they can rarely be relied upon to fully resolve a situation of anovulatory anoestrus at the herd level, unless attention is also paid to alleviating the predisposing factors.

The most common causes of anovulatory anoestrus that can be managed at a farm level are those relating to nutrition and stress. When micronutrient deficiencies exist in the absence of significant underfeeding, correction of the deficiency can produce a relatively rapid response. More commonly, effects of trace element deficiency are compounded with energy deficiency and, when energy deficiency is the primary cause of anoestrus, it is unlikely that a rapid response will be achieved by suddenly increasing energy intake (Rhodes et al. 2000). Stimulation of ovarian activity usually requires 3 to 4 weeks of improved feeding before a response occurs. When poor responses are obtained, it is normally possible to identify a time when feeding was suboptimal.

Hence, improvement of overall energy levels in the diet usually has to be part of a long-term strategy for the farm. Management of the dry period has to be improved so that cows calve in the optimum condition score. Management of the transition cow has to be improved so that she does not suffer from hypocalcaemia, and her rumen is prepared to respond to postcalving changes in nutrition. Management of feeding has to be improved, ensuring that supplies and requirements are as closely matched as possible, that feeding regimens are used that will ensure maximum dry matter intakes, and that all age groups of animals have equal (or, in the case of first-calvers, preferential) access to food. Most of these principles apply equally to dairy and beef cows, although the opportunities for modifying feed management practices of dairy cows are usually much greater than for beef suckler cows. In beef suckler cows, temporary weaning and restricted suckling, together with the use of progestogens (see later in this chapter) during the time of calf removal have resulted in reducing the time to the first ovulation postpartum.

Alleviation of other predisposing factors, such as stress, can be difficult. Avoiding mixing groups of cows at critical times seems obvious, yet management of high- and low-yielding groups of cows can make this difficult to achieve in practice. Provision of adequate feeding space is more easily achievable, but reducing group sizes to numbers in which cows can establish normal social hierarchies can be very difficult to achieve. The effects of climatic stress can be reduced by the provision of shade to cows that are heat stressed. Provision of shelter to cows that have to stand around in cold, draughty collecting yards can also be remarkably effective. The incidence of lameness can be almost entirely attributed to management practices, so anoestrus due to this cause should be regarded as being due to poor management rather than an inherent pathological problem.

Hormonal Treatment

The principles of the hormonal treatments for the management of anoestrus are described in Chapters 8 and 22.

Many different hormonal treatments have been given to anoestrous cattle in attempts to cause a resumption of cyclic activity. The main regimens that have been developed are based either on progestogens, relying on the so-called 'pituitary rebound' effect to restart the cycle, or upon drugs that either cause the release of gonadotrophins or have direct gonadotrophic actions themselves. The principles that underpin successful treatments for anoestrus (Wiltbank et al. 2011) are to:

1. Ensure that cows are cycling at the end of the voluntary wait periods or at the start of the breeding season, to maximise the chance of a timely conception;
2. Control ovarian follicle wave development to ensure that the ovulatory follicle is mature but not atretic;
3. Control the timing of ovulation to allow the use of fixed time AI or to maximise the opportunities for effective detection of oestrus; and
4. Ensure that the induced corpus luteum is functionally normal in terms of the duration of its persistence and the magnitude of its progesterone secretion.

There are currently four major methods of treating anoestrus:

- Ovsynch (GnRH–$PGF_{2\alpha}$–GnRH) (see Chapter 8);
- Ovsynch plus intravaginal progesterone;

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- Intravaginal progesterone plus eCG (and/or GnRH, $PGF_{2\alpha}$); and
- Oestradiol plus intravaginal progesterone plus (and/or GnRH, $PGF_{2\alpha}$).

These regimens meet the four principles for controlling ovulation in anoestrous cows. The first administration of GnRH or oestradiol esters causes ablation of dominant follicles, either by ovulation or luteinisation *in situ* (GnRH) or regression (oestradiol). Thereafter, a new follicular wave starts, in response to the FSH surge that is elicited by the administration of GnRH and/or the loss of the dominant follicle. Progesterone, which in dairy cows is most conveniently administered by the intravaginal route (but can be administered as an implant in beef animals), has two effects: suppression of ovulation of the growing follicular wave and accumulation of LH within the pituitary. Withdrawal of progesterone and/or administration of $PGF_{2\alpha}$ causes regression of the CL and permits ovulation. The final GnRH ensures synchronous ovulation. In animals that are deeply anoestrous, eCG can be given at the time of progesterone removal as a means of augmenting the growth of the preovulatory follicle (Bryan et al. 2013). Although eCG has long been used in deeply anoestrous beef cows, it is now increasingly also being used in dairy cows. Treatment regimens that use oestradiol cannot be used in the EU or in animals supplying food products to the EU, but oestradiol can still be used in many other areas, including North and South America. Fixed time insemination is used in nearly all anoestrus treatment regimens in order to obviate the need for oestrus detection and, more importantly, because the majority of cows either do not display oestrus at all or do not do so in close association with the optimal time for insemination. Several studies have shown better conception rates to fixed-time insemination than to detected oestrus. These regimens are summarised in Fig. 22.23.

Many studies have shown that the responses to any of the foregoing regimens are better in animals that are well fed rather than poorly fed. Most evidence shows that the response is better in animals whose plane of nutrition approaches equilibrium rather than being in energy deficit (e.g., McDougall et al. 2005).

Resynchronisation programmes are sometimes used as a means of managing the cows that fail to conceive after treatment for anovulatory anoestrus, but then relapse into anoestrus. The latter cows are frequently assumed to be pregnant. These cows, together with cows that spontaneously relapse into anoestrus, sometimes called 'phantom cows' (Cavalieri et al. 2000a, b), present considerable problems for herd managers as they either fail to conceive or have a very long open period. Management of such animals is aimed at preventing their relapse into anoestrus from going undetected. A further placement of intravaginal progesterone-releasing inserts after breeding (various studies have replaced them 12–16 days after breeding) will allow positive identification of cows that have failed to conceive.

Cystic Ovarian Disease

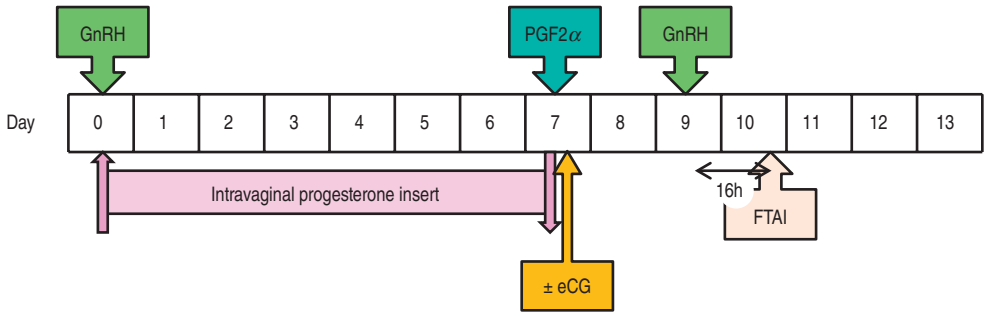
Ovarian cysts are follicular structures resulting from ovulation failure that are frequently larger than normal and persist within the ovary for longer than normal follicles, resulting in aberrant reproductive function. A cyst is defined as being larger than 25 mm in diameter if single (Al-Dahash & David 1977b), but, when multiple cysts are present, they can be smaller (> 17 mm diameter) (Garverick 2007). The definition normally requires the absence of a functional CL (Youngquist 1986).

In the normal postpartum cow, follicular waves start soon after calving, with ovulation of the dominant follicle of one of the early waves. Cystic ovarian disease (COD) is the clinical manifestation of dysfunction of this process, arising as a result of anovulation of a dominant follicle. Sakaguchi et al. (2006) suggested that the development of cysts is symptomatic of a more generalised disturbance of ovarian function, which ranges from a series of anovulatory follicle waves of normal duration, through temporary appearance of anovulatory cystic structures that develop and regress in a cyclical manner, to anovulatory cysts that persist in the ovary for extended periods of time (Fig. 22.24). Hence, many cows develop large, fluid-filled structures in the ovaries during the immediate postpartum period. These normally regress spontaneously but present as a clinical problem when they cause aberrant behaviour or alterations of the oestrous cycle.

Prevalence

Surveys of the prevalence of cystic ovarian disease (or COD) need to be interpreted with some caution because information on the effects of the cysts upon reproductive function is usually absent from abattoir surveys, whereas in herd studies, cysts should only be regarded as pathological if they persist into the breeding period. Frequencies found in abattoir surveys range from 0.5% (Australia: Summers 1974) to 18.5% (Japan: Fujimoto 1956). Al-Dahash and David (1977a) found a prevalence of 3.8% in a British abattoir survey.

Figures for the prevalence of COD ranging from 6% to 19% per lactation were reported from the United States (Day 1991), 5% from Canada (Bigras-Poulin et al. 1990), and 7% to 8% from the UK (Watson 1996). Youngquist (1986) estimated the incidence at between 10% and 20% but considered that, if the cysts that occur before the start of the breeding season are also taken into account,



• **Fig. 22.23** Basic treatment regimen for treating anoestrus. The basic GnRH–$PGF_{2\alpha}$–GnRH is augmented with intravaginal progesterone and, sometimes, with eCG. Insemination is at a fixed time after the last GnRH (FTAI).

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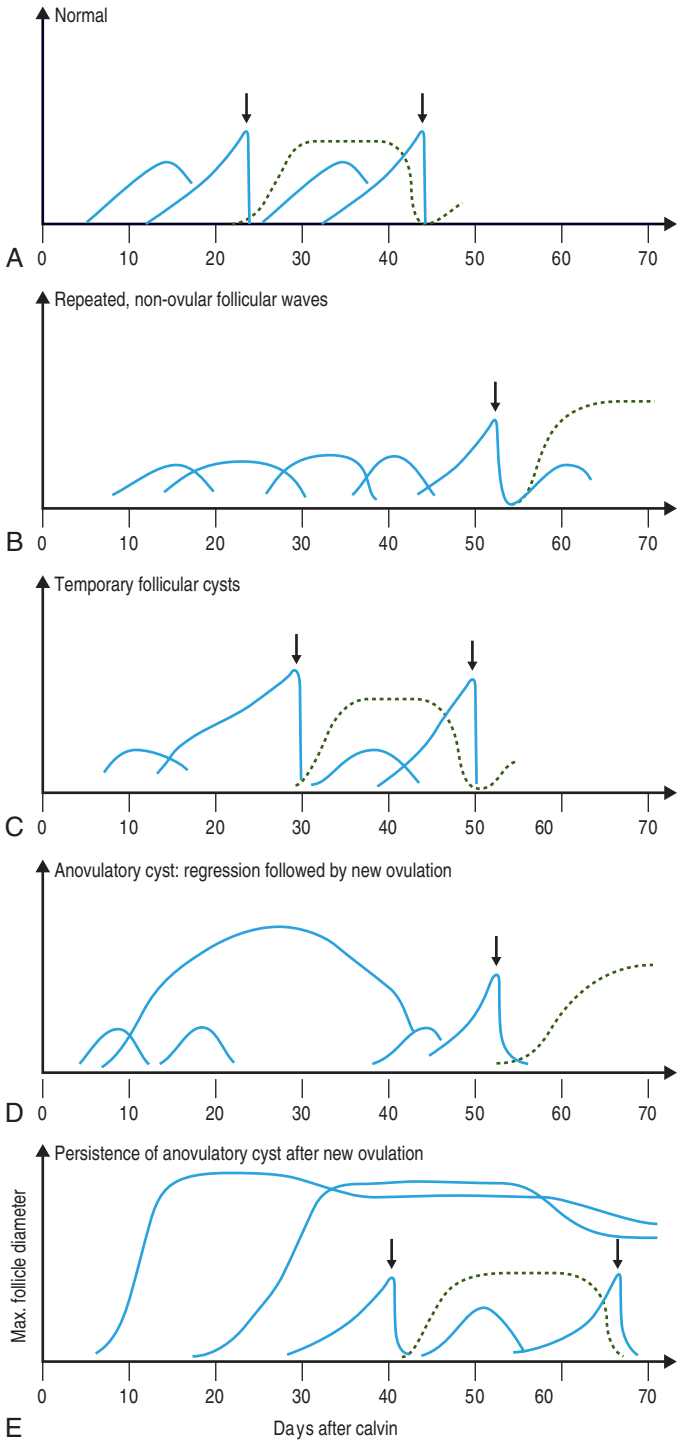

• **Fig. 22.24** Sequences of postpartum follicle growth in a cow that (A) is normal; (B) has repeated non-ovulatory follicles; (C) has short-term follicular cysts; (D) has cystic ovarian disease; and (E) has persistent ovarian cyst after new ovulation. (Adapted from Sakaguchi et al. 2006.)

the incidence is about 30%. Anecdotal evidence has frequently suggested that cystic ovarian disease is increasing in incidence, but this is difficult to quantify from the literature.

Aetiology and Pathogenesis

In cows with COD the preovulatory LH surge is absent or attenuated (Peter 2004), resulting in failure of ovulation of the dominant follicle. The consequence of failure of ovulation is that the follicle persists as a cystic structure, either with maintenance of the granulosa ('follicular cyst') or with luteinisation *in situ* in the absence of ovulation ('luteal cyst').

After calving there is a progressive restoration of the ability of the hypothalamus to generate an LH surge in response to oestradiol (Kesler & Garverick 1982), as a part of the normal process of resuming oestrous cycles. Cows with COD have a partial or complete failure of oestradiol to elicit a normal, positive feedback, LH surge (Nanda et al. 1991). Thus there is a lack of sensitivity of the hypothalamic surge-generating centre to oestradiol (Garverick 2007) and a failure of GnRH release. On the other hand, there is no lack of hypothalamic GnRH content, nor a lack of pituitary LH content, nor a lack of pituitary responsiveness to LH (Vanholder et al. 2005). Abnormalities of FSH secretion do not appear to be involved in the disorder (Hamilton et al. 1995). There are several factors that contribute to the lack of sensitivity to oestradiol. First, if a GnRH/LH surge occurs at a time when no follicle capable of ovulation is present, the hypothalamus becomes unresponsive to the positive feedback effects of oestradiol (Gülmen et al. 2002), which also occurs if there is no rise in progesterone after an ovulation. Responsiveness of the hypothalamus has to be restored by progesterone (Gülmen & Wiltbank 2005). Second, the LH surge mechanism can be attenuated by stress. Inhibition of ovulation and cyst formation (Liptrap & McNally 1976) and an impairment of the LH surge mechanism, similar to that in cows with COD (Dobson et al. 2000), can be produced experimentally by exogenous adrenocorticotrophic hormone (ACTH) or hydrocortisone. These changes can also be induced by stresses that activate the hypothalamo–pituitary–adrenocorticoid (HPA) axis (Nanda & Dobson 1990). Third, Diaz et al. (2015) have found that it is possible to replicate the condition of COD by maintaining progesterone concentrations slightly above baseline for 5 to 15 days after the expected time of ovulation. Because the inhibitory effect of elevated concentrations of progesterone on the LH surge is well-recognised, subluteal concentrations of progesterone could well be involved in the pathogenesis of COD.

The second major factor in the development of COD is alterations in the biochemical activity of the follicle. First, there is evidence (Kawate et al. 2004) of a reduced population of LH/chorionic gonadotrophin receptors (LHCGR) in the granulosa of cysts compared with normal follicles, which may be attributed in part to the effects of the HPA axis, because ACTH administration impairs steroid synthesis and LH expression within the dominant follicle (Biran et al. 2015). There is also a reduced concentration of oestrogen receptors in the granulosa cells of cysts (Salvetti et al. 2007) and alterations in postreceptor regulators of steroidogenesis (Ortega et al. 2015). Other recent research has identified differences in several autacoids or locally active hormones between normal and cystic follicles. Diaz et al. (2016) found differences in expression of bone morphogenetic proteins (BMP4 and BMP6) and the BMP receptor 1B (BMPR1B) between cystic and normal follicles (BMP4 and BMP6 are important regulators of steroidogenesis and mitosis in granulosa cells) that may contribute to follicular persistence and disruption of local hormone production. Expression of cytokines such as Interleukins (IL-6 and IL-8) and tumour necrosis factor–α (TNFα), all of which have roles in steroidogenesis and ovulation, differ between normal and cystic follicles (Stassi et al. 2017). Likewise, expression of transforming growth factor–β (TGFβ), a regulator of cell replication and steroidogenesis within the ovary, also differs between normal and cystic follicles

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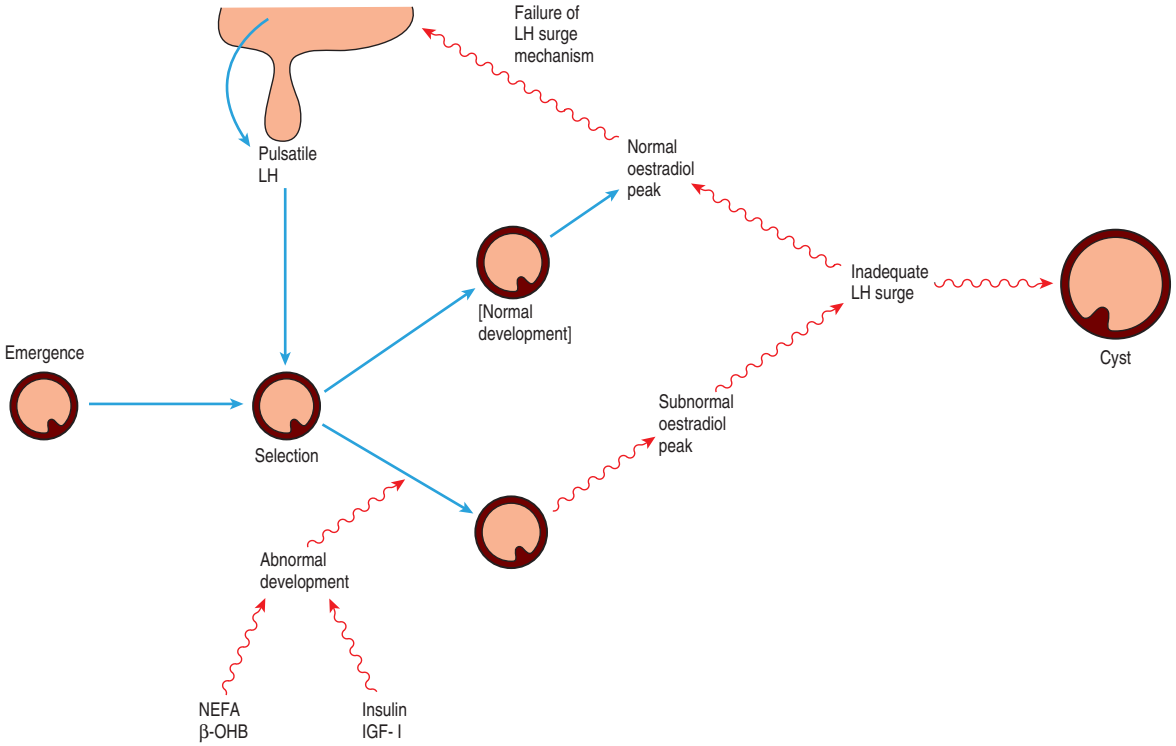

• **Fig. 22.25** Schematic representation of the pathogenesis of ovarian cysts. (Redrawn from Vanholder et al. 2005.)

(Matiller et al. 2014). All of these differences in autacoid activity may contribute to follicular persistence and ovulation failure.

Negative energy balance is associated, at an epidemiological level, with COD, and there may be direct negative effects of elevated non-esterified fatty acids (NEFAs) upon follicular function. High yielding dairy cows have, as a result of genetic selection and metabolic load, low concentrations of insulin and IGF-I, and there is considerable evidence that these may contribute to the development of COD through an impairment of follicular cell proliferation and steroidogenesis (Vanholder et al. 2005). Rodriguez et al. (2017) also found alterations of the IGF system, such that cystic follicles had greater expression of IGF receptor 1 (IGFR1) and IGF binding protein 4 (IGFBP4) than normal follicles.

A scheme for the aetiology of COD based on that proposed by Vanholder et al. (2005) is shown in Fig. 22.25.

Cows with COD also have a higher frequency and amplitude of LH secretion during cyst growth than do normal cows (Cook et al. 1991). It was originally suggested that these abnormal patterns are causal of COD (Farin & Estill 1993), but current opinion is that, although such patterns of LH secretion are effective at maintaining a cyst once it has developed (Hampton et al. 2003), they are not required for the formation of cysts. On the other hand, it has been suggested that the abnormalities of tonic LH secretion are a reflection of the presence of progesterone concentrations that are marginally above baseline values (Vanholder et al. 2005).

Predisposing Factors

Cystic ovarian disease arises as an interaction between a hereditary predisposition, stress, milk yield, age, and plane of nutrition.

- **Hereditary predisposition:** There are differences in incidence of COD between breeds and between families and sires within a breed, which indicate the likelihood of an inherited predisposition. Estimates of heritability are variable but generally low (Zwald et al. 2004a, b). Because of the familial basis of the condition, its incidence can be dramatically reduced within a breed by avoiding the use of cows that have had COD as bull mothers and by avoiding the use of bulls that have bred daughters that have the disease as bull sires (Bane 1964, Kirk et al. 1982).
- **Breed:** COD is more common in Holstein–Friesians than in other dairy breeds. It is rare in beef breeds.
- **Age:** The incidence of COD is greatest in animals between 4 and 6 years of age (Roberts 1986). It has traditionally been regarded as being uncommon in animals in their first lactation, although Watson (1998) showed that the incidence in first lactation animals was similar to that in second and third lactations.
- **Yield and nutrition:** Many studies have linked COD to milk yield (e.g., Ashmawy et al. 1992). However, the relationship with yield is complex, as it involves the extent of negative energy balance, the genetic potential of the cow (see previous mention), and the metabolic load of lactation. Thus milk yield per se may not be as significant a cause of cystic ovarian disease as is the genetic potential for high yield (Zulu & Penny 1998, Hooijer et al. 2001). Body condition at drying off has also been associated with the disease (Gearhart et al. 1990), presumably through effects on postcalving fat mobilisation. Excessive dietary protein may also have a direct effect on the incidence (Ashmawy et al. 1992). Hooijer et al. (2001) found that there was a genetic correlation between COD and milk production of 0.34 and between COD and milk protein production of 0.44.
- **β-carotene:** It has been suggested that β-carotene may reduce the incidence of COD (Lotthammer 1979). However, this has

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not been supported by the work of others (Folman et al. 1979, Marcek et al. 1985), who found no benefit from such supplementation of cows' diets.

- **Season:** Some studies have found that COD is more common in autumn and winter than at other times of the year (Nelson et al. 2010, Cattaneo et al. 2014).
- **Intercurrent disease:** Ketosis, dystocia, stress, twin births, RFM, milk fever, and postpartum uterine infections have also been considered risk factors (Hardie & Ax 1981, Roberts 1986. Bosu & Peter 1987, Laporte et al. 1994).

Clinical Presentation

Cysts may be present in one or both ovaries (Table 22.2). More cysts are identified in the right ovary than in the left, reflecting the relative activity of the two ovaries. Solitary cysts are more common than multiple cysts, although the proportion of cows reported with solitary cysts varies widely (75%: Elmore et al. 1975; 53%: Al-Dahash & David 1977b). The majority of cysts are between 2.5 and 3.0 cm in diameter, with very few larger than 5 or 6 cm.

TABLE 22.2 Distribution of ovarian cysts and their association with corpora lutea

Type of Cyst	Number of Cysts	PERCENTAGE OF CYSTS: No Corpus Luteum Present	PERCENTAGE OF CYSTS: Corpus Luteum Present	Total (%)
Thin-walled	Single	16.9	18.9	80.2
	Multiple	40.3	4.2	
Thick-walled	Single	10.2	7.7	19.8
	Multiple	1.9	0	
Total		69.3	30.7	

From Al-Dahash & David (1977b).

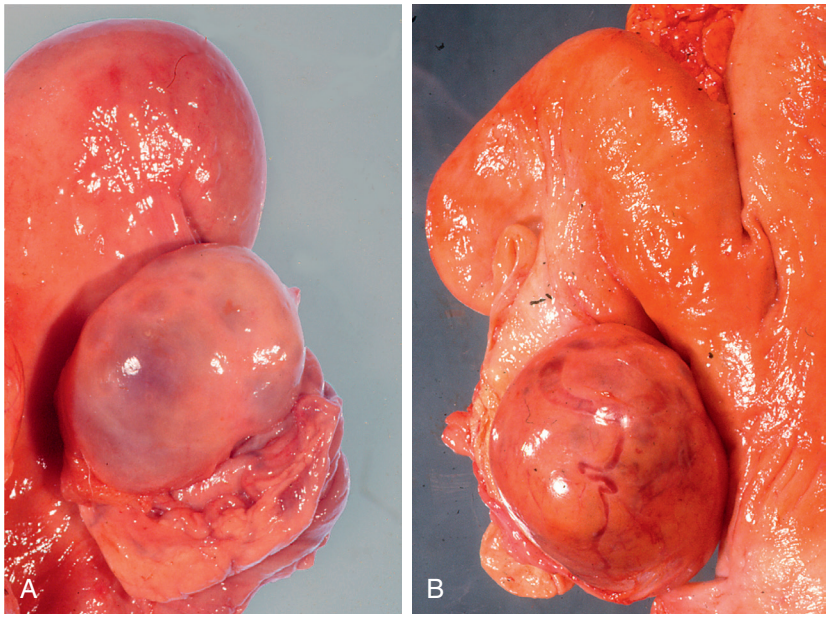
Follicular cysts are two or three times more common than luteal cysts (Leslie & Bosu 1983).

Ovarian cysts can be classified as either follicular or luteal. Follicular cysts are thin-walled, fluctuating structures that can be single or multiple (Fig. 22.26A). Luteal, or luteinised, cysts (see Fig. 22.26B) are thick-walled and are usually single (Table 22.3). Identification and differentiation by ultrasonography (Fig. 22.27A and B) is generally more accurate than by manual palpation (Douthwaite & Dobson 2000). Although useful, this binary classification does not account for the many cysts that are partly luteinised or for changes in the extent of luteinisation over the lifespan of a cyst, so care is needed in interpreting clinical findings. There are a number of other fluid-filled structures that may be present on the ovary and need to be differentiated from cysts. The corpus haemorrhagicum, adjacent luteal and follicular structures, non-ovarian cysts (e.g., parovarian cysts, hydatids of Morgagni), abscesses, and tumours can also be confused with cysts (Farin & Estill 1993). The type of cyst that is present can be confirmed by measuring peripheral progesterone concentrations; blood or milk progesterone concentrations: higher than 2 ng/mL in milk or higher than 1.0 ng/mL in plasma/serum (Carroll et al. 1990, Farin et al. 1992) are considered to be indicative of a luteal cyst.

Cysts can be present concurrently with a corpus luteum (Table 22.2; Al-Dahash & David 1977b, Roy et al. 1985, Carroll et al. 1990), so the absence of a corpus luteum needs to be confirmed. However, in cows that also have a corpus luteum, any cysts that are present are likely to be hormonally inactive (Al-Dahash & David 1977c) because of loss of the granulosa cells and so are considered as anatomical remnants rather than active structures.

Clinical Signs

The main clinical signs of COD in cattle are persistent oestrus, anoestrus, or masculinisation; it was first discovered as a result of investigations of persistent oestrus behaviour in cows. Roberts (1955) described 'nymphomaniac' cows as displaying excessive, prolonged signs of oestrus, with oedematous swelling of the vulva, frequent and copious discharge of clear mucus, and a shortened interval between successive heats. In neglected cases, there is nervous



• **Fig. 22.26** (A) Thin-walled follicular cyst and (B) thick-walled luteal cyst in cows' ovaries.

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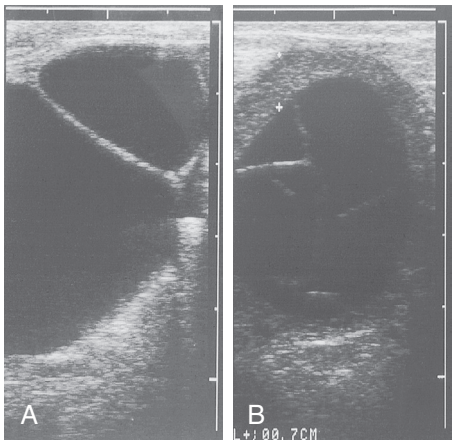
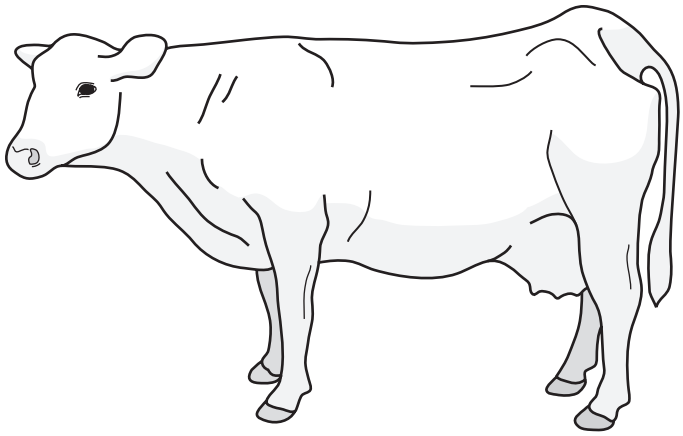
TABLE 22.3 Fluid-containing structures in bovine ovaries

NORMAL STRUCTURES		ABNORMAL STRUCTURES		
Follicles	**Vacuolated Corpora Lutea**	**Follicular Cysts**	**Luteinised Follicles**	**Luteinised Cysts**
Transient, dynamic, soft, fluctuant structures, which reach 1.5–2.0 cm in diameter just before, during and for 12 hours after oestrus	Same size as non-vacuolated CL but may feel slightly softer on palpation. Evidence of ovulation point. Central vacuole disappears during pregnancy	Soft, thin-walled, fluid-filled structure ≥ 2.5 cm diameter that persists. Thickness of cyst wall < 3 mm	< 2.5 cm diameter. No evidence of ovulation point. Larger cavity than vacuolated corpus luteum	Thick-walled, fluid-filled structure ≥ 2.5 cm diameter that persists. Thickness of cyst wall > 3 mm
Follicles ≤ 1.5 cm diameter are normally present at all stages of oestrous cycle	Usually single	May be single frequently multiple, in one or both ovaries	Usually single	Usually single
	Occurs after ~25% of normal ovulations	Follow anovulation of mature follicle	Follow anovulation of mature follicle	Follow anovulation of mature follicle
Associated with normal oestrous cycle	Associated with normal oestrous cycle	Associated with low peripheral progesterone concentrations. Affected cows are either anoestrous or nymphomaniacal	More likely to occur in immediate postpartum period. Associated with normal or shorter length of oestrous cycle	Associated with high peripheral progesterone concentrations. Affected cows are anoestrous



• **Fig. 22.27** Ultrasound images of the ovary of (A) a cow with a thin-walled cyst and (B) a luteal cyst; note thick wall of luteal tissue, which is >3 mm in thickness. (Courtesy WR Ward.)



• **Fig. 22.28** Cow with the typical nymphomanic conformation. Note the raised tailhead and the thickening of the neck and brisket.

behaviour, depressed milk yield, loss of bodily condition (even emaciation), sinking of the sacrosciatic ligaments, and upward displacement of the coccyx (Fig. 22.28). Affected cows attempt to ride other cows and will stand to be mounted by other cows. Because of their excessive sexual activity they have a generally disruptive effect upon the rest of the herd, making accurate oestrus detection difficult. Occasionally, cows with long-standing COD will become virilised (Arthur 1959).

Most cows with COD are, however, anoestrous. Watson (1998) found that only 2% of cows with COD were presented for frequent, irregular display of oestrus, compared with 74% that were presented as being anoestrous. A further 12% were found during routine prebreeding examinations, and 12% were found in cows that were non-pregnant at pregnancy diagnosis. Likewise, in the series of cases reviewed by Roberts (1986), 63% to 85% of animals were anoestrous. The behaviour of cows with cysts is largely unrelated to peripheral steroid concentrations (Roberts 1986); cows with follicular cysts have blood oestrogen (Kittock et al. 1973, Dobson et al. 1977) and testosterone (Eyestone & Ax 1984) concentrations that are not greatly dissimilar from those of normal cows.

Sequelae

Spontaneous recovery from cystic ovarian disease occurs frequently in the early postcalving period, although it is difficult to estimate this figure accurately in the light of the data, which show that cysts can be short- or long-term structures (e.g., Sakaguchi et al. 2006; Fig. 22.24). Reported self-cure rates vary from 5% to 30% of cases (Garverick 1997, Peter 2004) within 45 days of calving. If divided into cysts that arise from the first dominant follicle versus those that arise from subsequent waves, the self-cure rates are 50% and 20% respectively (Kesler & Garverick 1982). Neglected cases of COD can result in mucometra, in which there is distension of the uterus with mucoid fluid and thinning of the uterine wall (Fig. 22.17; Al-Dahash & David 1977a).

Cystic ovarian disease results in an extension of the calving interval. Cows with follicular and luteal cysts had calving to conception intervals of 274 and 408 days, respectively, compared with 115

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days in unaffected animal (Chamba et al. 2017). Pregnancy rates of cows with COD (67%) are also lower than in normal cows (89%) (Cattaneo et al. 2014). Hence, cows with COD show decreases in lifetime milk yields and increases in the involuntary culling rate, and are more likely to become repeat breeders (Moss et al. 2002). Nonetheless, in both the field study of Watson and Cliff (1996) and the computer modelling of Scholl et al. (1990), it was found that, although COD has a major effect upon reproductive outcomes of individual animals, the condition was not of major significance in determining the reproductive performance at the herd level. Thus Watson (1998) calculated that the net effect of COD within a herd was to increase the mean calving to conception interval by approximately 2 days and the number of serves per conception by 0.1 to 0.2 serves. For the individual animal, treatment is economically worthwhile; de Vries et al. (2006) calculated a net benefit of approximately US$80 of treatment with a progesterone insert compared with no treatment.

Treatment

Physical Methods. It is generally advocated that cysts should not be manually ruptured per rectum, as this can cause trauma, haemorrhage, and/or ovariobursal adhesions (see Nanda et al. 1989). Despite the known risks and limited efficacy of manual rupture, Watson (1998) reported from a survey of field practice that nearly 35% of cysts were 'treated' by manual rupture (although half of these also had hormone treatment). More recently, aspiration of the contents of ovarian cysts has been suggested as a treatment (Amiridis 2009), but this does not seem to offer much advantage over hormonal treatments.

Hormonal Programmes. Most cysts are treated using reproductive hormones. The choice of hormonal treatment regimen depends largely upon the type of cyst that is present. Follicular cysts are usually treated with either gonadotrophic hormones (i.e., hCG or GnRH) or progesterone, whereas luteinised cysts can be treated with luteolytic substances.

Follicular Cysts. Follicular cysts can be treated with hCG or GnRH. These hormones do not cause ovulation of the cyst; rather, they cause ovulation of new follicles (Jeffcoate & Ayliffe 1995), which develop into corpora lutea. Treatment may also cause luteinisation of the cyst *in situ.* Lower doses of GnRH (100–250 μg) probably cause luteinisation of the cyst (Kesler et al. 1981); higher doses (e.g., 0.5–1.0 mg GnRH or 10 μg buserelin) probably cause ovulation of new follicles and formation of a corpus luteum (Bertchtold et al. 1980). The result is an increase in progesterone concentration, which causes negative feedback on LH secretion and resets the sensitivity of the pituitary to oestradiol (Gülmen et al. 2002). Thus LH concentrations decline, and there is a decline in follicular steroid synthesis, leading to lowered oestradiol–17β concentrations (Kesler & Garverick 1982). The luteinised structures produce progesterone for 15 to 18 days (Garverick 2007) so that there is a return to normal oestrus in approximately 3 weeks.

Resumption of normal oestrous cycles occurs within 3 weeks in 65% to 90% of cows after treatment with GnRH (Dobson et al. 1977, Probo et al. 2011), and in 75% of cows after treatment with hCG (Dobson et al. 1977). Conception rates to the next oestrus range from 37% to 55% (Ijaz et al. 1987). However, when the response to treatment, proportion of cows inseminated, and the conception rate to AI are considered together, the response to treatment is relatively poor. Probo et al. (2011) found that these figures were 71%, 70%, and 40%, respectively, giving a pregnancy rate after treatment of only 20%. Cysts recur in 8% (Nanda et al. 1988) to 16% (Watson 1998) of cows after treatment.

Once luteinisation has occurred, the interval to the next oestrus can be shortened by giving $PGF_{2\alpha}$ (Garverick 2007). Thus, Kesler et al. (1978) and Garverick (1997) suggested giving GnRH on the day of diagnosis of the cyst and $PGF_{2\alpha}$ 9 days later. However, studies (e.g., Archibald et al. 1991) of subsequent fertility compared with that achieved after using GnRH alone did not show any benefit of the addition of $PGF_{2\alpha}$. Ismail et al. (2017) found that a greater proportion of cows exhibited oestrus after GnRH + $PGF_{2\alpha}$ than after GnRH alone (87% versus 70%) but that the final pregnancy rate after three insemination cycles was identical (63% and 65%).

GnRH–$PGF_{2\alpha}$–GnRH ('Ovsynch', 'GPG') regimes (see Chapter 8) have been used to treat COD (Ambrose et al. 2004, Garverick 2007). Gundling et al. (2015) compared the results of a standard ovsynch protocol (Day 0: GnRH; Day 7: $PGF_{2\alpha}$; Day 9: GnRH) with a modified protocol (Day 0: GnRH + $PGF_{2\alpha}$; Day 14: $PGF_{2\alpha}$; Day 16: GnRH), finding that the reproductive performance of cows treated with the modified protocol was similar to that of unaffected controls in terms of display of oestrus, first service conception rate, and final pregnancy rate, but the standard ovsynch protocol gave significantly poorer results over all of those parameters. They calculated an economic benefit of the modified protocol of €132 over the standard ovsynch regimen. Similarly, Freick et al. (2012) found that only 19% of cows with COD became pregnant after a standard Ovsynch protocol, whereas 35% became pregnant after a similarly modified protocol. Thus there appears to be limited benefit to the more expensive ovsynch regimen over a simple GnRH or GnRH + $PGF_{2\alpha}$ protocol, but the modified programme may be cost effective in terms of reproductive outcomes.

Alternatively, follicular cysts can be treated with intravaginal, progesterone-releasing inserts (Nanda et al. 1989, Calder et al. 1999). Increased peripheral progesterone concentrations result in a lowering of pulsatile LH secretion and a restoration of the ability of the hypothalamo–pituitary axis to generate an LH surge in response to oestradiol (Gumen & Wiltbank 2002, Todoroki & Kaneko 2006). Signs of persistent oestrus abate within 24 hours of PRID (progesterone releasing intravaginal device) insertion, the cysts gradually regress and, after removal after 9 to 12 days, there is oestrus with ovulation and corpus luteum formation. Treatment of cows with follicular cysts with PRID alone resulted in similar calving to conception intervals to untreated cows without COD, although 40% of treated cows were eventually culled for failure to conceive (Douthwaite & Dobson 2000). Several reports have found progesterone alone to be a relatively ineffective treatment for luteinised cysts (e.g., Douthwaite & Dobson 2000, Todoroki et al. 2001), so there needs to be careful differentiation of the type of cyst before initiation of treatment or incorporation of a luteolytic agent into the programme. Mollo et al. (2012), on the other hand, found similar results after GnRH, hCG or progesterone treatment of cows with COD, regardless of whether they had follicular or luteal cysts. A comparison of the responses to GnRH, PGF, and intravaginal progesterone is given in Table 22.4 (Watson 1998).

Luteinised Cysts. The most logical way to treat a luteal cyst is the use of $PGF_{2\alpha}$. A consistent response was obtained by Dobson et al. (1977): 26 of 27 cows showed regression of the cyst, the majority came into oestrus in 3 to 5 days, and 56% of the cows conceived, at a mean treatment-to-conception interval of 27 days. Jackson (1981), in a survey involving several countries, reported over 80% response with disappearance of the cyst and oestrus within 3 to 5 days, with at least 60%, and in most cases over 90%, of these cows conceiving. Many similar reports exist in the

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TABLE 22.4 Comparison of GnRH, $PGF_{2\alpha}$ and intravaginal progesterone for treating cystic ovarian disease

Treatment	Interval From Calving to First AI (d)	Conception to First AI (%)	Calving - Conception Interval (d)	Overall Pregnancy Rate (%)	Services Per Conception (n)	Interval From Start of Treatment to Conception (d)	Success (Treated Cows That Conceived) (%)
Cows With Cystic Ovarian Disease							
No treatment	76	35	124	77	3.5	50	78
GnRH	88	41	151	79	3.6	55	81
$PGF_{2\alpha}$	82	46	126	85	2.4	50	89
Progesterone	78	32	131	78	3.5	59	85
Normal Cows From the Same Herds							
	76	45	101	NQ	2.2		

NQ, not quoted.
Derived from Watson (1998).

literature (see Ijaz et al. 1987). Indeed, failure of cows with supposed luteal cysts to respond to $PGF_{2\alpha}$ therapy is most commonly due to misdiagnosis.

Prevention

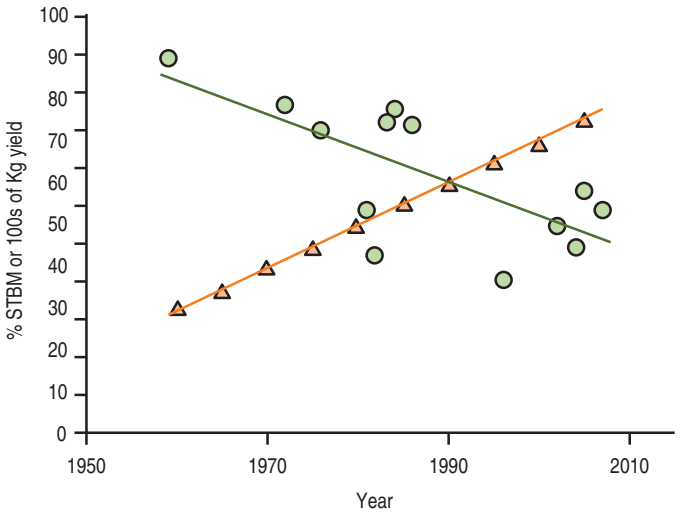
By careful genetic selection, improvements have been made by eliminating bulls that have sired daughters that have subsequently suffered from COD. Ideally, cows should not be treated for cystic ovaries, and their progeny certainly should not be used for breeding. Unfortunately, this places the herd manager and the veterinarian in a dilemma because, frequently, those cows that are affected are the highest yielders.

Prophylactic use of GnRH (e.g., 100–200 μg of GnRH 12–14 days postpartum; Kesler & Garverick 1982) has shown some success in reducing the prevalence of cysts in herds (Zaied et al. 1980). However, Noseir et al. (2013) found no difference between treated and untreated cows in terms of conception rates, services per conception, and calving to first service interval.

Expression of Oestrus

Cows display oestrus behaviour in the absence of a bull by mounting and standing to be mounted by other sexually active cows (see Chapter 1). The relatively short duration of oestrus is one of the main reasons that observation has to be undertaken both regularly and frequently. Early reports indicated that oestrus can last for 18 to 20 hours (Tanabe & Almquist 1960), but most studies have found it to last no more than 15 hours (Esslemont 1974, Albright & Arave 1997), and that 20% of cows are in oestrus for less than 6 hours. Until recently, the consensus view has been that most normal cows exhibit oestrus behaviour at the appropriate stage of the cycle and that, provided appropriate observations were undertaken, oestrus detection rates in excess of 90% should be achievable (Williamson et al. 1972).

More recently, there has been evidence for a decline in the intensity of oestrus behaviour in high yielding dairy cows, with a general decline in the number of mounts, the duration of each mount, and the length of time over which mounts occur. Dobson et al. (2008) reviewed literature on the duration and intensity of oestrus, showing that the proportion of cows that stand to be mounted has declined from nearly 90% in the 1950s to around 50% in the 2000s (Fig. 22.29). This decline in intensity of oestrus display has been accompanied by a reciprocal increase in the proportion of cows having 'silent heats'. The assumption that 100% of cows in physiological oestrus will show the classic behavioural signs of standing to be mounted during oestrus therefore no longer appears to be true for high yielding dairy cattle. In the study of Kerbrat and Disenhaus (2004), in which continuous video monitoring of cows was compared with milk progesterone profiles (as an indicator or physiological oestrus), only 8 of 15 cows ever stood to be mounted during the periovulation period. Other recent studies have confirmed that the reduction in intensity and duration of oestrus behaviour has resulted in low detection rates using standard observational techniques. van Vliet & van Eerdenburg (1996) only observed standing oestrus in 37% of cows, whereas Dransfield et al. (1998) recorded that 24% of cows had an oestrus period that was of low intensity and short duration, compared with only 8% with an oestrus of high intensity and long duration. Sveberg et al. (2015) compared the duration and intensity of oestrus in Holstein versus Norwegian Red cows, showing that the Norwegian Red cows



• **Fig. 22.29** Percentage of animals standing-to-be-mounted (STBM ◯) and average milk yield (△) in Holstein–Friesian dairy cows reported for 50 years to 2008. (Based on Dobson et al. 2008.)

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displayed more oestrus behavioural episodes, had a longer duration of oestrus, and were associated with 'sexually active groups' (SAGs) for longer than Holstein cows. Interestingly, in that study, the reduction of oestrus behaviour was an effect of breed rather than of yield per se, inasmuch as the yields of the Holsteins and Norwegian Reds were identical.

The reduced intensity of oestrus behaviour is explained in part with reference to preovulatory oestrogen concentrations. Although Glencross et al. (1981) considered that intensity of oestrus behaviour was unrelated to oestrogen concentrations, more recent studies (e.g., Lopez et al. 2004) have found that preovulatory oestrogen concentrations are lower in high yielding than low-yielding cows and that the intensity of behaviour is related to those oestrogen concentrations (Lymio et al. 2000, Lopez et al. 2004). In other words, high yielding cows have a lower oestrogen concentration and a reduced intensity of oestrus behaviour. The extent to which lower oestrogen concentrations are due to insulin- and IGF-I–mediated deficiencies of follicular growth (Butler & Smith 1989, Lucy 2001) or to the increased metabolism and clearance that occurs in cows with a high metabolic load (Sangsritavong et al. 2002) is yet to be established. There may also be changes in the central nervous system that are unrelated to altered steroid profiles (Mayne 2007).

Follicular or Luteal Dysfunction

Inadequate luteal function, although not a cause of anoestrus, is an ovarian dysfunction that results in a reduction in fertility. Many aspects of early embryonic development are progesterone dependent, such that inadequate progesterone concentrations are associated with impeded embryonic development. Thus, for example, inadequate expansion of the trophoblast can result in failure of maternal recognition of pregnancy, and hence luteolysis occurs. Furthermore, some cows can undergo premature luteolysis before the time of maternal recognition of pregnancy (Meier et al. 2009).

Persistent Corpus Luteum

Anything that interferes with the production or release of prostaglandin $F_{2\alpha}$ will result in a persistent corpus luteum (CL). Pregnancy is the condition that most frequently results in persistence of the CL. However, as previously described, in the presence of uterine infection and inflammation of the tissues, there is interference with the production or the release of $PGF_{2\alpha}$ (Seguin et al. 1974). This condition can be self-perpetuating because progesterone domination of the uterus reduces its resistance to infection, and prevents a return to oestrus (with its associated increase in uterine immunity). There is, however, evidence that persistence of the CL can occur in the absence of uterine lesions. Lamming and Bulman (1976) identified that 2% of cows have milk progesterone concentrations that are elevated for more than 30 days. Others have associated luteal persistence with failure of ovulation or follicular development.

If it is considered that a persistent CL is present, it can be readily treated with $PGF_{2\alpha}$ or a synthetic analogue, provided, of course, that the clinician is confident that the cow is not pregnant.

Ovulatory Defects

Ovulation in the cow normally occurs 25 to 35 hours after the onset of behavioural oestrus and 18 to 26 hours after the ovulatory LH peak (see Chapter 1). Extended intervals between the onset of oestrus and ovulation ('delayed ovulation') of up to 48 hours have been reported in dairy cows: typical incidences range from 2% to 5% (Erb et al. 1976, Roelofs et al. 2005). Concentrations of oestradiol and LH in the preovulatory period of such cows may be lower than in the normal follicular phase (Bloch et al. 2006). Conception rates of cows with delayed ovulation are generally low.

Some studies have associated delayed ovulation with subnormal progesterone concentrations in the after luteal phase (Bloch et al. 2006). On the other hand, some cows apparently have a prolonged follicular phase of the oestrous cycle, as determined by the presence of low progesterone concentrations in blood and milk (Erb et al. 1976, Bulman & Lamming 1978), that is not due to delayed ovulation but to delayed onset of progesterone secretion by the CL.

Stockmanship and Management

Oestrus Detection

The use of AI as the main method of breeding dairy cows means that the primary responsibility for oestrus detection falls upon the staff who manages the herd. Oestrus detection is therefore a critical aspect of dairy herd management for, although good oestrus detection does not necessarily guarantee good reproductive performance, poor oestrus detection makes poor performance hard to avoid.

Whether or not the behavioural signs of oestrus are observed depends on many factors (Table 22.5), and, notwithstanding the changes in the expression of oestrus that are occurring in high yielding cows, there is much that can be done at a management level to optimise the efficiency and accuracy of its detection. For example, the timing and duration of observations can be optimised and the signs that are indicative of oestrus behaviour can be recorded (Heres et al. 2000, Kerbrat & Disenhaus 2004), and technological aids can be used to improve the efficiency of oestrus detection. As difficulties in detecting oestrus have increased, the use of whole-herd synchrony techniques, which bypass the need for human detection of oestrus, has increased (see Chapter 8). Conversely, in herds that are naturally mated, the importance of oestrus detection is less critical, largely being confined to ensuring that adequate non-return rates occur after service.

Herd Size and Calving Pattern

In order for effective display of oestrus, there must be cows that are prepared to mount as well as cows that are prepared to be mounted. Cows that mount are generally those that are coming into or going out of oestrus, and these, together with the cows that are in oestrus, associate together in a sexually active group (SAG). Cows that are in dioestrus or anoestrus are not generally present in the SAG.

It is clear that the duration and intensity of oestrus increase as the number of animals in the SAG increases (Hurnik et al. 1975, Esslemont et al. 1980, Kilgour & Dalton 1984), making oestrus detection easier in a larger group. Systems that have strongly seasonal calving and mating patterns generally have much larger SAGs than year-round-calving herds and, consequently, better oestrus detection. In year-round-calving herds, only approximately 2% of the cows calve within any given week. In consequence, there will be times when the sexually active 'group' consists of a single cow, making oestrus detection very difficult indeed (van Vliet & van Eerdenburg 1996, van Eerdenburg et al. 2002, Kerbrat & Disenhaus 2004).

Herd size affects the detection of oestrus in two main ways. First, as herd size (and concentration of calving pattern) increases, the size of SAGs increases, making oestrus detection easier. On the other hand, there is a reduction in the accuracy and efficiency of oestrus detection associated with increased herd size (Esslemont

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TABLE 22.5 Factors associated with efficiency of oestrus detection

Time Allowed for Oestrus Detection
How much?
How often?
When, in relation to the activity patterns of the cows?
Human Social Factors
What other activities are happening on the farm at the same time?
What other pressures exist for the herdsman's time?
Is the detector able to recognise the signs of oestrus?
Is the detector interested in detection of oestrus or is it 'just another job'?
Calving Pattern
How many cows?
How many cows in the sexually active group?
For how much of the year does oestrus detection have to be undertaken?
Housing
Are cows housed or at pasture?
Is there room for cows to display oestrus behaviour?
Can cows that are not in oestrus avoid being ridden?
Identification and Records
Can cows be identified accurately?
Are cows observed for returns to oestrus after artificial insemination?
Aids
Tail paint, heatmount detectors, electronic aids, etc.?
Relative reliance placed on aids and primary observation?

1974, Wood 1976). Negative effects of increasing herd size are that the herd manager has to look after more cows, so cows tend to lose their individual identity and, consequently, are neither so accurately identified nor so closely observed (Waiblinger & Menke 1999). Likewise, there is evidence for impaired display of oestrus in herds that are too large or have groups that change too frequently for a stable social hierarchy to develop between individual cows.

Time and Place of Observation

There are times and situations when cows exhibit few or no signs of oestrus behaviour and others when the display of oestrus is more likely to occur. Cows are unlikely to display oestrus behaviour when being held in collecting yards, during milking, when feeding, or when moving along races or tracks. Crowded collection areas and confined spaces may not permit the ready grouping of sexually active individuals. Conversely, cows are more likely to display oestrus in cubicle yards, in feeding or 'loafing areas', or when at pasture (Williamson et al. 1972, Esslemont & Bryant 1976, Pennington et al. 1985, 1986).

Tie-stall housing has a significantly adverse effect upon the display of oestrus (Unal et al. 1986, Felton et al. 2012), such that fewer oestrus periods are detected, although with no diminution upon accuracy of detection. Systems in which cows are continuously in tie-stalls through all or part of the year (e.g., Scandinavian herds: Refsdal 1999; small-holder herds in developing countries: Abeygunawardena et al. 2000) are therefore at particular risk of poor oestrus detection.

Several studies have reported that the floor surface is of importance in the display of oestrus behaviour. Concrete surfaces are more slippery than dirt surfaces and hence less conducive either to mounting or to being mounted (Britt et al. 1986; Vailes & Britt 1990), and individuals that have previously slipped on concrete may be unwilling to attempt to mount in the future (Albright 1994). Thus the majority of mountings take place in conditions that have the best footing and are less crowded (Albright & Arave 1997, Pennington et al. 1985). The detection of oestrus is better in cows that are pastured than in those that are housed (Palmer et al. 2010), regardless of whether visual observation or automated detection methods are used.

It has long been considered that cows are more likely to display oestrus behaviour during the night than during the day. Curiously, when video or radiotelemetry have been used to determine patterns of mounting, this nocturnal pattern has not been evident (Amyot & Hurnik 1987, Nebel et al. 2000). Indeed, Albright and Arave (1997) concluded that the results of studies of the timing of oestrus behaviour were inconsistent with simple diurnal patterns and probably should be explained in terms of the periods of time for which cows were left undisturbed by other farm activities. Slightly more ovulations occur at night than during the day (56% versus 44%: Kahn 1992), but relatively few between 0900 and 1500 hours. Perhaps these data could be interpreted to mean that the prevalence of ovulations and oestrus behaviour that occurs during the night may simply be an indication of the time when there is least other activity on the farm (see also Williamson et al. 1972, Hurnik et al. 1975, Esslemont & Bryant 1976).

Esslemont (1973) examined the effect of the use of a rigid regimen, involving three or four periods of observation for 15 or 30 minutes, upon oestrus detection efficiency. With three 15-minute periods of observation (0800, 1400, and 2100), a detection rate of 69.6% was achieved. Increasing the duration to 30 minutes improved the rate to 81.2%, and four 30-minute periods of observation (0800, 1400, 2100, and 2400) produced the best result of 84.1%. The absolute times of observation are not critical and can be varied to suit the timetable of the farm. van Vliet and van Eerdenburg (1996) suggested that, if oestrus is observed when cows are in a space in which they can form sexually active groups and when they are undisturbed (i.e., after completion of milking and feeding undisturbed), at a time when dioestrous cows are resting and cudding, two observations of 30 minutes per day would result in a detection efficiency of 74% and an accuracy of 100%.

Identification

It must be possible to readily identify the individual cow from any position so that the herd manager can then record the animal number immediately and permanently. Good freeze branding on the rumps, together with numbered collars or large ear tags, should preferably be used for identification. Even in small herds, whose stock managers think that they know the cows as individuals, many mistakes are made through misidentification or misrecording. When observation is made of housed cows, provision of adequate lighting is required for cows to be seen showing behavioural signs and for their accurate identification.

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Environmental Factors

Patterns of oestrus behaviour vary with environmental temperature, with the frequency of mounts, and with the repertoire of activities differing between hot, moderate, and cold conditions (Pennington et al. 1985). In cold conditions, mounting behaviour was much less between 1800 and 0600 than at other times of day, whereas in hot conditions it was least during the hottest part of the day. Also, the duration of oestrus was reduced to 8 to 10 hours under extreme climatic conditions. Certainly, where autumn calving and winter mating is practised (as in the UK), a shortening of the duration of oestrus and a reduction in the intensity of oestrus behaviour are widely recognised as problems of mating management of such herds.

Human Factors

Despite the importance of good oestrus detection to the economic performance of the dairy herd, it is often regarded as a 'chore' that has to be fitted in between the many other tasks that have to be done. Therefore perhaps it is not surprising that, in many situations, oestrus detection efficiency is poor. On the other hand, when oestrus detection is regarded as a priority task and is undertaken by highly motivated farm staff under ideal conditions for observation, oestrus detection rates can be remarkably high. An important prerequisite for effective oestrus detection is therefore the provision of enough time to do the job properly (O'Connor 2007).

With the general decline in numbers of staff working on dairy farms and the difficulties in getting junior staff that have any experience of working with livestock, there is often a dearth of personnel who are capable of accurate oestrus detection. O'Connor (2007) emphasised the importance of making sure that one person is assigned responsibility for oestrus detection and ensuring that the person is adequately trained to do so. The importance of having staff who are trained to identify oestrus correctly was shown by Esslemont (1974), who found that there was a significantly higher oestrus detection rate by trained staff compared with untrained staff. The importance of having staff who 'fully understand the signs of heat and are fully committed to heat detection in an optimal management' (Becker et al. 2005) has not diminished with the passing of time. When there is a defined breeding season, it is possible to use the detection of early (preseason) oestrus periods both as a means of retraining staff in the skills of oestrus detection and of ascertaining which cows are still anoestrous. Prebreeding oestrus detection also allows prediction of the timing of the first oestrus of the mating period. Recent changes in the intensity of behavioural oestrus implies that personnel need to be able to recognise a range of relatively subtle behaviours, as well as the classic sign of standing to be mounted (Heres et al. 2000, Lyimo et al. 2000, Kerbrat & Disenhaus 2004).

The length of the breeding season affects the ability of herd managers to detect oestrus. When there is a short breeding season, detection rates can reach 90% or more, because of a combination of a concentration of staff effort on detection of oestrus and large SAGs. In year-round calving herds, the dispersal of effort and the small SAGs mean that oestrus detection rates are rarely better than 60%, and in many cases, they are often much less than that.

'Silent' Ovulations

Most cows do not display oestrus at the time of their first postpartum ovulation because of a lack of progesterone priming (King et al. 1976). For the reasons discussed previously, it is increasingly common for cows to have low intensity of oestrus behaviour over a short period of time, which may not include standing to be mounted. Hence these cows may also be considered to have 'silent ovulation', even though at least some behavioural signs associated with oestrus will generally have been displayed.

Other causes of inadequate display of oestrus behaviour include heat stress (Gwazdauskas et al. 1983). Cows suffering from ergotism ('fescue toxicity') may become suboestrous, whereas a number of nutritional deficiencies, including β-carotene, phosphorus, copper, and cobalt, are also said to cause suboestrus.

However, the most common cause of 'silent ovulation' is a failure of oestrus detection. Hence, once convinced that cows are not suffering from anovulatory anoestrus and that there are no pathological causes of suboestrus, herds with a high incidence of 'silent ovulation' generally benefit from either a thorough review of oestrus detection procedures or the use of detection aids such as are described here.

Aids to the Detection of Oestrus

There are several methods available that can be used to improve the oestrus detection rate. Traditionally, these have been regarded as 'aids', inasmuch as they have been adjuncts to human observation of oestrus behaviour. With the difficulties presented by reduced intensity of behavioural oestrus display and the declining pool of labour with livestock skills, recent research has concentrated on attempting to fully automate detection aids, with the intention of obviating the need for human detection. Some of the aids to oestrus detection are summarised in Table 22.6.

Detection of Mounting

Detection of mounting can be achieved by marking the tailhead with paint so that when the cow is mounted the marking is disturbed, or by placing a pressure-sensitive device on the tailhead to record the act of mounting.

Tail Painting

The technique of tail painting was developed in New Zealand (Macmillan & Curnow 1977) to improve oestrus detection rates. A brittle, high gloss, enamel paint is placed as a thick layer in the midline over the sacrum and base of tail (Fig. 22.30A), preferably applied using a brush against the line of the hair to ensure good adhesion, before smoothing in the direction of the hairline. There should be regular inspection of the paint so that repainting can be done if necessary. When a cow is mounted by other cows, the paint is abraded and removed (see Fig. 22.30B). Submission rates of over 90% and pregnancy rates of up to 60% can be obtained after AI after observation of the condition of the tail paint. Some inaccuracies (positive or negative) of detection occur when between 25% and 75% of paint remains (Kerr & McCaughey 1984), although these can be minimised by concurrent direct observation for behavioural signs of oestrus. At the time of writing, the use of tail paint is almost ubiquitous in Australia and New Zealand. Tail paint has been used in the UK but has proved to be of little overall benefit to oestrus detection rates. This may be because of the small SAGs that occur in non-seasonal herds.

Heatmount Detectors

Simple mechanical heatmount detectors such as the Kamar or Bulling Beacon can be used. These consist of a soft, translucent plastic dome attached to a rectangle of canvas in which there is placed a soft plastic vial of red dye that is fixed with adhesive just cranial to the base of the tail (Fig. 22.31A). When a cow is mounted and the vial is subjected to sufficient pressure (i.e., at

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TABLE 22.6 Aids to the detection of oestrus in cows

Method	Use	Advantages	Disadvantages
Based Upon Mounting Behaviour			
Tail paint	Paint applied over the tailhead is removed when cows are mounted by each other	Improves results from visual detection alone	Needs considerable experience to decide if cows with partially removed paint are actually in oestrus
Heat mount detectors (e.g., Kamar, Bulling Beacon)	Capsule applied over the tailhead is broken when the cow is mounted, turning the capsule red	Gives a more clear cut result than tail paint in cows that are only mounted a few times	Capsule can burst by rubbing, or if a cow is unable to avoid a single mount when she in not in oestrus
Oestrus detection patches (e.g., Estrotect)	Patch applied over tailhead; removal of surface layer reveals a bright under–colour when the cow is mounted.	Gives a more clear-cut result than tail paint, but fewer false positives than capsules	Missed oestrus events can occur when devices are not checked or are rubbed in cubicles.
Electronic heatmount detectors (e.g., Heat watch)	Applied over tailhead; records mounts electronically	Accurate provided cows stand to be mounted	Relatively expensive, as it requires specialised equipment
Teaser bulls	Chin bull marker shows which cows have been mounted	Positive indication of oestrus	Presence of intact bulls in the herd, bulls become disinterested with time, small risk of spreading venereal disease
Based Upon Increased Activity			
Pedometers	Increased walking activity is measured as an indicator of oestrus	Improves results above that commonly achieved by visual detection	Relatively expensive, as it requires specialised equipment; moderate sensitivity and specificity
Neck-mounted activity monitors (e.g., Heatime) (Aungier et al. 2012)	Increased movement activity is measured as an indicator of oestrus	Improves results above that commonly achieved by visual detection	Relatively expensive, as it requires specialised equipment; moderate sensitivity and specificity
Based on Physiological Changes			
Method	**Use**	**Advantages**	**Disadvantages**
Milk progesterone	Monitor cycle and do fixed time AI when concentration falls	Very accurate if done carefully	Expensive; inaccurate if not done carefully
Vaginal mucous resistance	Daily monitoring of vaginal resistance	Independent of behavioural signs	Invasive
Milk composition and temperature	Inline measurement during milking	Fully automated, so minimises requirement for human observation	High capital input; validation of results is not complete

Based on Parkinson et al. (2010).

• **Fig. 22.30** Tail paint applied as an aid to oestrus detection (A) after application to a group of cows over the caudal sacral and tailhead areas and (B) after having been rubbed off by mounting during oestrus. (Courtesy Dr. Jakob Malmo.)

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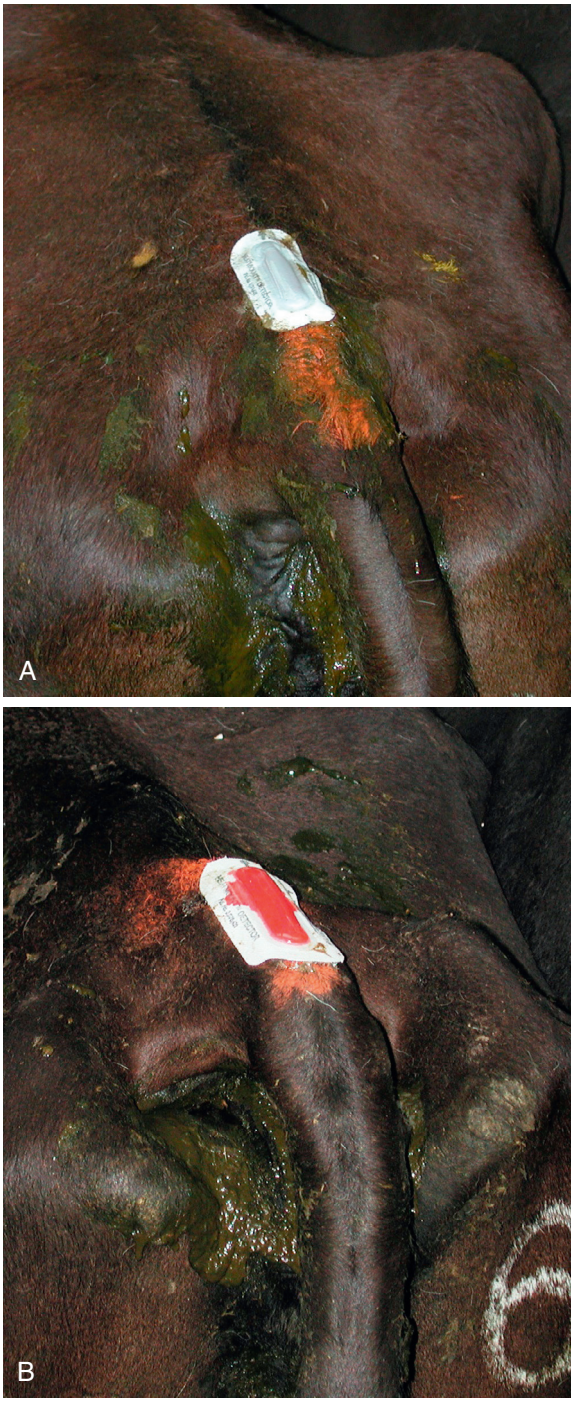

• **Fig. 22.31** Kamar heatmount detector attached to the sacrum of a cow (A) before activation and (B) after activation. (Courtesy Dr. Jakob Malmo.)

standing oestrus), it is compressed, the dye escapes, and the dome becomes red (see Fig. 22.31B). False positives can occur when the detector is activated by a cow rubbing the underside of a rail, or in crowded collecting yards when a cow that is not in oestrus cannot escape the attentions of mounting cows. Detectors can also become detached when placed on wet coats or when the winter coat is being shed. Despite these caveats, however, heatmount detectors are widely used, with first-service conception rates of more than 60% having been reported (Phatak & Touchberry 1988). Holman et al. (2011) showed that the sensitivity of oestrus detection by Kamars alone was identical to that achieved by farm staff observation, but that the sensitivity was increased when the two methods were combined, with the sensitivity increased to 76%. There is some evidence to suggest that heatmount detectors have benefits over tail paint when the intensity of oestrus behaviour is low (i.e., as tail paint relies on multiple mountings for its removal).

Alawneh et al. (2006) reported the use of a novel oestrus detection strip that consists of a reflective material covered by a low sheen, black acrylic paint. The strip is applied to the sacrum, and when the cow is mounted, the black covering is rubbed off. The reflective strip can then be observed via an overhead camera. First-service pregnancy rate was approximately 70% in cows in which the detection strip was used versus approximately 40% in cows in which oestrus detection was by observation plus the use of tail paint. Various commercial variations on this method (e.g., Bonato et al. 2012) have produced similar results. A comparison of such 'scratch cards' with visual observation and other forms of heatmount detectors is given in Table 22.7.

Radio–telemetric heatmount detectors are available as an alternative to visual inspection of heatmount detectors (e.g., HeatWatch, ShowHeat). In such systems, an electronic pressure-sensing system is linked via a radio–telemetric link to a computer data analysis system, which sorts the information by cow and generates activity lists (e.g., oestrus, possible oestrus, etc.) (Nebel et al. 1995, Walker et al. 1995, Dransfield et al. 1998). Oestrus detection rates of more than 90%, with accuracies of approximately 95%, have been recorded using this system (Albright & Arave 1997, Cavalieri et al. 2000a), a figure that is as good as the best that can be achieved by behavioural observations and is very much better than national averages for oestrus detection. Timing of insemination may need to be altered after the use of heatmount detectors (i.e., compared with timing after direct observation). Dransfield et al. (1998) suggested an optimal interval of 4 to 12 hours after the first mounting episode, with a significant diminution of pregnancy rate after 16 hours.

Activity Monitors

Cows' movement and locomotor activity increases during oestrus, which can be used as the basis for its detection (Kiddy 1977, Schofield 1990).

Pedometer devices typically contain a mercury switch that is activated by the movement of the cow. They are attached either to one hind leg or, more recently, to the neck. The locomotor activity of individual cows depends not only upon the stage of the cycle but also upon the size of the SAG (Varner et al. 1994), with the result that pedometer systems are more accurate at detecting oestrus in groups of animals than in individuals (Roelofs et al. 2005). The accuracy is also affected by the interface between the pedometer and the data analysis system, because infrequent extraction of data from the pedometer (e.g., twice a day at milking time) is less effective than real-time acquisition of data (Lyimo et al. 2000, Nebel et al. 2000). Thus the accuracy of pedometers is greatest when activity data is interpreted by a computer algorithm in relation to the 'background' level of activity of the individual animal (Eradus & Braake 1993). Felton et al. (2012), however, found that the benefits of pedometers are very much reduced in cows housed in tie-stalls compared with those that have freedom of movement. Saint-Dizier and Chastant-Maillard (2012) therefore concluded that the performance of pedometers for oestrus detection may be affected by the environmental conditions, particularly as walking activity of dairy cows varies according to the type of housing and the herd management system. In pasture-based systems, in which

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TABLE 22.7 Oestrus detection practices and detection rates in dairy herds

SYNCHRONISED OESTRUS, UK HERDS: HOLMAN ET AL. (2011)		
Detection Method	**Sensitivity (%)**	**Positive Predictive Value (%)**
Visual detection	56.5	92.9
Scratchcard	35.9	63.9
Kamar	56.7	61.3
Pedometer	63.3	73.5
Neck-mounted activity monitor	58.9	93.5

RESYNCHRONISED OESTRUS, AUSTRALIAN HERDS: CAVALIERI ET AL. (2003)		
Detection Method	**Sensitivity (%)**	**Positive Predictive Value (%)**
Tail paint	91.3	91.7
Kamar	85.7	95.9
Heat Watch	84.4	100
Pedometer	81.4	87.5

SPONTANEOUS OESTRUS, GERMAN HERDS: GAUDE ET AL. (2017)		
Detection Method	**Oestrus Detection Rate (%)**	**Reliability Rate (%)**
Neck-mounted activity monitor	85.0	91.8
Visual detection	52.2	94.6

SPONTANEOUS OESTRUS, AUSTRALIAN (SEASONALLY CALVING) HERDS: MORTON (2000A)	
Detection Method	**% Of Herds Having High (> 94%) Heat Detection Rates**
Neither paddock checks nor HMD used	32
Paddock checks but not HMD used	50
HMD but not paddock checks used	59

HMD, heatmount detectors; Heat Watch: automated heatmount detector.

cows walk different distances on different days due to pasture rotation, the interpretation of pedometer data requires different parameters to those for housed cows.

Collar-mounted activity monitors (e.g., Heatime) assess the movement of the head and neck and are being used to detect oestrus. The system consists of an acceleration sensor, microprocessor, memory, infrared detection unit, and central computer processor. The central processor compares the current average activity level to baseline activity levels to identify increases in activity. Significant increase in activity (e.g., 5 x s.d. above baseline) is interpreted that the individual cow is in a preovulatory follicular phase, but the detection accuracy depends on appropriate setting of duration and activity thresholds. Saint-Dizier and Chastant-Maillard (2012) reviewed the relationships between increased activity, time of ovulation, and fertility. Ovulation takes place on average 29 to 33 hours after the onset of increased activity and 17 to 19 hours after the end of increased activity in lactating Holstein cows (Roelofs et al. 2005, Hockey et al. 2010). Aungier et al. (2012) optimised the interpretation of data from spontaneously cycling cows, to give 87.5% correct identifications of oestrus and 21.3% false positive identifications (see also Table 22.7). However, insemination to oestrus detected by activity monitors did not a give better conception rate in a Presynch–Ovsynch protocol than fixed-time AI (Burnett et al. 2017); however, the proportion of cows detected in oestrus by activity monitoring was substantially greater than that detected by visual observation (Gaude et al. 2017).

Conductivity of Vaginal Fluids

At oestrus the resistance of vaginal mucus falls in association with the rise in oestrogen concentrations. Various attempts have been made to use this as a means of predicting oestrus, although with generally disappointing results (Foote et al. 1979, Cavestany & Foote 1985). The variability in the measurements may well be related to the fact that the tip of the probe with its associated electrode is not in contact with vaginal mucus. Kitwood et al. (1993) reported that the position of the probe within the vagina affects impedance readings. When regular examination of cows can be backed up with a computer-based data-logging and analysis system, results are better than when 'one-off' measurements are made because, although most cows exhibit decreases in resistance at oestrus, most individuals differ in their baseline resistance during dioestrus. Hence the measurement of relative changes in resistance may be more useful than measurement of absolute values. Rezac et al. (1991) found that lowest impedance values could occur on Day –1, 0, or +1, relative to the day of oestrus (Day 0), although HongXiang et al. (2017) were able to predict the timing of the onset of oestrus and of ovulation with reasonable accuracy from the nadir of vaginal mucous resistance.

The main practical limitation to the use of vaginal probes is that it is labour intensive, requiring daily probing of each cow and cleaning and disinfection between cows. Experimental implantation of sensors beneath the vulval mucosa has been attempted to circumvent these problems (Lewis et al. 1989) but, although results appear promising, no further development of this technique has been reported.

'The Mechanical Nose'

Development of gas-sensing systems and work on the identification of pheromone-secreting cells in the perineum of oestrous cows (e.g., Blazquez et al. 1994, Lane & Wathes 1998) make it possible that direct electronic sensing of the odours of oestrus pheromones, perhaps as cows pass a detector during milking, may be feasible within the foreseeable future. At present, difficulties of sampling appear to be the main factor limiting the adoption of such technology.

Inline Progesterone Measurement

The decline in blood and milk progesterone concentrations at the end of the luteal phase precedes ovulation by a relatively constant interval. Milk progesterone concentration is therefore a reasonably good indicator of impending oestrus. Non-automatic progesterone measurement is not readily amenable to continuous monitoring of concentrations, but automatic systems have been developed. Delwiche et al. (2001) reported successful use of an in-line biosensor to measure milk progesterone concentrations

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as an indicator of oestrus, and, subsequently, automated systems have become available (e.g., Herd Navigator, DeLaval, Sweden) which measure progesterone concentrations at every milking. In addition to predicting oestrus, inline progesterone measurement can be used to assist with pregnancy diagnosis, detecting embryo losses and the presence of ovarian cysts (Friggens & Chagunda 2005, Bruinje et al. 2017). Birgersson (2013) studied the effects of the Herd Navigator system upon the fertility of Swedish and Dutch dairy herds: there was a significant reduction in the calving to first insemination interval, and a trend towards shorter calving interval. In addition, farmers found that the system reduced the time that needed to be spent undertaking oestrus detection.

Indirect Detection

Use of Teaser Bulls, Androgenised Steers or Cows

Vasectomised or other sterile entire or androgenised steers can be used, either equipped with some form of marking device (e.g., a chin-ball marker) or in association with 'heatmount' detectors. Gordon (2006) found that the use of vasectomised bulls increased the submission rate for AI from 61% to 69%, and reduced the calving-to-first service and calving-to-conception intervals (74 versus 85 days and 115 versus 120 days, respectively) while having no effect on conception rate. Despite these benefits, vasectomised bulls have not been very popular in the UK, largely because of perceived difficulties of managing bulls that are running loose with the herd. Furthermore, when venereal diseases are present, they represent a major health hazard because of their ability to transmit such diseases.

Penile deviation can also be used as a means of preparing sterile bulls. For this, the preputial orifice is freed from its normal attachments and is relocated some distance from the midline. Although this procedure is not permitted in the UK, it is considered to be an effective aid to the detection of oestrus in many other countries. Its disadvantages include those common to the use of gonad-intact bulls. Moreover, some bulls learn how to serve despite the penile deviation, whereas others desist from mounting at all. These and a number of other surgical procedures for creating marker bulls are described by Morgan (2007).

Androgenised cows can also be effective for detecting oestrus (Britt 1980). By administering testosterone propionate in oil by intramuscular injection every week for 3 weeks, a suitable teaser is prepared, which can be used from about 2 weeks after the last injection. Maintenance of sexual activity requires repeat treatment at intervals, but these androgenised cows have distinct advantages because they are safer and do not transmit venereal disease.

Video Monitoring

Continuous video recording can be made of the areas where cows are housed, which, so long as there is adequate lighting and good animal identification, can be used to identify cows that are in oestrus. Although the results of continuous video monitoring can be very good indeed, a means of automating the recognition of oestrous cows needs to be used (Roelofs et al. 2005) for significant time savings to be achieved.

Accelerated viewing or observation of only those cows which are moving may be methods to improve the speed of reading image data. Bruyere et al. (2012) described the use of conventional and infrared cameras, which were linked to computer analysis to record only those periods in which cows were moving. Using this system, the oestrus detection rate was greater than for visual observation (80% versus 69%), with similar positive predictive values (93%–94%).

Use of Oestrus Synchronisation and Induction Programmes

Because of the difficulties of maintaining effective levels of oestrus detection, whole-herd oestrus synchronisation programmes have been developed as a means of circumventing the need for human observation. These programmes, whether based on sequential administrations of prostaglandin $F_{2\alpha}$, GPG programmes, or more complex presynchrony–synchrony–resynchrony regimens, all aim to replace insemination to detected oestrus with fixed-time insemination, although resynchrony programmes also aim to ensure that cows that have not conceived are reinseminated in a timely manner. These methods are described in full in Chapter 8.

Use of Bulls: 'Throwing in the Towel'?

A response of some farmers to all the foregoing difficulties, which is increasingly evident in the lay or farming press, is to retreat from the use of AI back towards the use of bulls. Advocates of this idea argue that it is better to have a cow that is pregnant to a mediocre bull than to have a cow that fails to conceive to a high-breeding-value bull. Likewise, they argue that it is better to breed cows by natural service than to require expensive pharmacological regimens whose outcomes in terms of per-service conception rates and of numbers of cows involuntarily culled for failure to conceive give no better results than natural service.

The main counterargument to this approach is the cessation of genetic advancement or even the loss of genetic merit for key productivity traits. It is also important to ensure that bulls are healthy (in terms of venereal disease, bovine viral diarrhoea, etc.), fertile, and managed safely.

Timing of Insemination

Faults of Detection

Milk progesterone profiles have been used to show that between 10% and 15%, or perhaps even 22%, of cows are inseminated during the luteal phase of the oestrous cycle (Hoffmann et al. 1974, Appleyard & Cook 1976). Clearly, such animals will fail to conceive. However, these figures do not include those animals that are inseminated during the follicular phase of the cycle at times that are not optimum for good conception rates. For example, Bulman and Lamming (1978) found that 15% of cows were inseminated during the luteal phase, but a further 15% were inseminated during inappropriate stages of the follicular phase.

The main reasons for these errors are incorrect identification of animals that are in oestrus and failure to appreciate the true signs of oestrus. Frequently, when large numbers of cows are inseminated at the incorrect time, the oestrus detection rate is poor, thus generally reflecting a poor standard of herd management. In such circumstances, some of the methods described previously should be used to improve the oestrus detection rate in the herd. In seasonally calving herds, the emphasis that is placed upon attaining high submission rates during the first few weeks of the mating period is such that many cows are presented for insemination that are not in oestrus. However, most of these cows are correctly identified a few days later, so the effect upon pregnancy rates is far less significant.

Optimal Timing of Artificial Insemination

Oestrus is short in the cow, with ovulation occurring 10 to 12 hours after the end of oestrus. During the next 6 hours the oocyte travels about a third of the way down the uterine tube, during which time fertilisation occurs, about 30 hours after the onset of oestrus (Robinson 1979, Roelofs et al. 2005). This figure has

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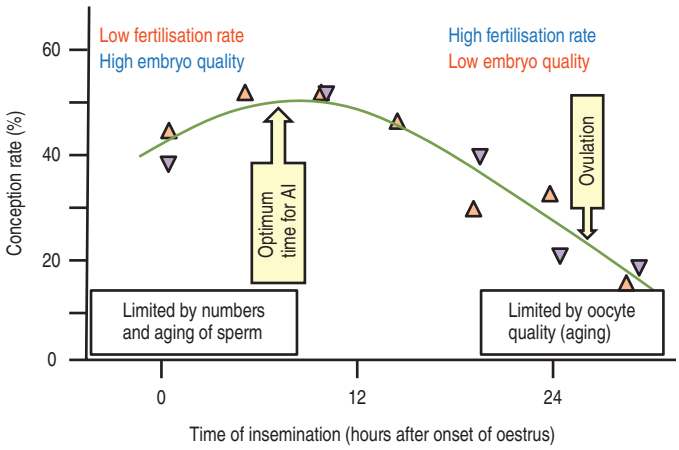

• **Fig. 22.32** Optimal timing of insemination in cattle derived from observations of the onset of oestrus (Trimberger 1948 ▽; Saacke et al. 2000 △) and as a theoretical compromise between fertilisation rate and embryo survival rate.

remained remarkably constant in view of the changes in oestrus behaviour that have occurred over the past 30 years, although some cows do exhibit significant prolongations of the interval between the onset of oestrus and ovulation (Bloch et al. 2006).

The best conception rates occur if insemination is carried out in the middle to the end of standing oestrus, i.e., 13 hours before to 8 hours before ovulation (Saacke et al. 2000, Nebel et al. 2000; Fig. 22.32). Traditionally, the recommendation for the timing of insemination has followed the a.m.–p.m. guideline, which is that cows observed in oestrus in the morning should be submitted for AI in the afternoon and cows observed in oestrus during the afternoon should be submitted for AI the next morning. Cows may conceive if they are inseminated at the beginning of oestrus or even 36 hours after the end of oestrus, but conception rates are reduced; if late insemination is undertaken, there is an increased risk of embryonic death. Nebel et al. (2000) suggested that, if the time of onset of oestrus is not precisely known, insemination should take place within 6 hours of its initial observation.

Nutrition

Nutrition and reproduction are highly interrelated. Plane of nutrition and growth rate exert substantial effects upon the onset of puberty, energy and protein feeding, and body condition are crucial in the resumption of oestrous cycles after calving, and there are a plethora of minerals, trace elements, and micronutrients that can have adverse effects on reproduction when they are deficient.

Puberty and the Rearing Period

Puberty, as determined by the appearance of the first oestrus, occurs between 5 and 20 months of age. It is considered that body size (rather than age), condition score, and plane of nutrition are the main factors that determine the age of puberty (Ducker et al. 1985, Spitzer 1986). In order to attain puberty, beef heifers should have reached 50%, and dairy heifers 35% to 45%, of their mature weight. Thus typical weights at puberty are 240 to 260 kg for Friesians, 270 to 300 kg for Herefords, and 230 to 250 kg for Angus. Within a group of cattle, however, puberty occurs at different body weights; for example, 50% of Hereford heifers exhibit oestrus at 272 kg body weight, whereas Charolais have to reach 308 kg for the same proportion of animals to be pubertal. For 90% of animals to be pubertal, the weights are 318 kg and 352 kg bodyweight, respectively (Spitzer 1986).

In most farming systems, it is preferable for heifers to calve for the first time at close to 2 years of age. For beef heifers, many studies from both the US and UK have found that beef breed heifers that first calve at 2 years of age are more profitable than those calving at 3 years (e.g., Nunez-Dominguez et al. 1991). In the study of Pinney et al. (1962), animals first calving at 2 years old produced 0.8 more calves per cow at 10% less cost over their entire production life than those first calving at 3 years old. Similarly, for dairy cows, the ideal age for first calving is approximately 24 months, as first lactation yield decreases rather than increases if the time of first calving is delayed (Spain et al. 2007). For seasonally calving dairy cows, both the age of first calving and the date of first calving are critical. Animals must calve right at the start of the season, which may mean that some calve at 23 or even 22 months of age. Attaining maximal growth rates during the rearing period is critical to achieve this (MacDiarmid 1999), both in terms of achieving adequate body size and ensuring that the youngest heifers have attained puberty at the start of the breeding season.

Age at puberty is therefore highly dependent on growth rate during the rearing period, which requires that animals be maintained on a high plane of nutrition. Gerloff and Morrow (1986) showed that the age at puberty of Friesian heifers decreases by 0.77 days for each additional 0.45 kg body weight at 6 months and by 0.36 days for each additional 0.45 kg body weight between 6 and 12 months of age. On the other hand, excessive growth rates of dairy heifers before puberty is associated with impaired development of the mammary epithelium and, as a consequence, reduced first lactation milk yields. Thus Foldager and Sejrsen (1982) suggested an optimal growth rate for Holstein heifers of approximately 600 to 700 g/day over the range 150 to 300 kg live weight. After puberty the effect of growth rate on mammary development is markedly lessened (Drew 2004).

These results demonstrate the importance of adequate feeding during calfhood to ensure the early onset of puberty. The aim should be to have calves that are healthy, with regularly monitored growth performance (Table 22.8) (McNeil et al. 2003, Schouten 2003), with planned growth rates to meet the target live weights at different stages of growth (Drew 2004). Generally, animals should have reached approximately 65% of their adult body weight at the start of the mating season in order to be pubertal and having regular oestrous cycles. Continued planned growth is then required to ensure that heifers have attained adequate body weight by the time of their first calving (Fig. 22.33). Heifers that have reached these targets should be able to reach their genetic potential for milk production in their first lactation, provided, of course, that their subsequent metabolic needs throughout pregnancy are not neglected. Live weight at calving has a significant effect upon subsequent reproductive performance: heifers that are underweight at their first calving have much lower conception rates and are more likely to be culled for infertility than those that are well grown (Table 22.9).

Nutrition and Reproduction in the Dairy Cow

Recent years have seen a dramatic decrease in the fertility of dairy cows. Many surveys have shown an inverse relationship (Fig. 22.1; Mann 2004) between fertility and yield (e.g., Nebel & McGilliard 1993, Washburn et al. 2002, Royal & Flint 2004, Butler 2005), which is generally considered to have resulted from: (i) selection for cows with the genetic potential for very high yields; and (ii) the metabolic demands of lactation. As a result, there has been an

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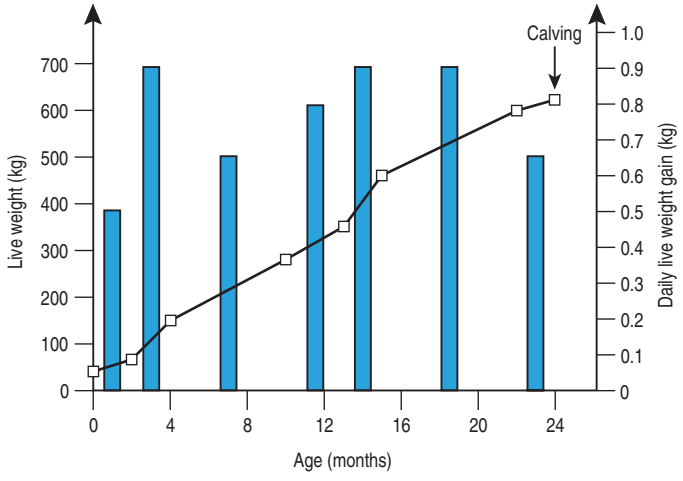
TABLE 22.8 Managing Heifer calves

Preweaning
Meticulous management of colostrum intake
Ensure good preweaning growth through well-managed milk feeding
Promote early development of the rumen by maximising early intake of concentrate feeds, provision of high quality roughage
Effective prevention of diarrhoea, respiratory disease, and navel ill
Postweaning
Weigh regularly to monitor growth rates adjust feeding as appropriate
Maintain high intakes of highly digestible forages
Avoid 'nutrient gaps', e.g., in dry summers or wet winters
Monitor regularly for trace element status
Effective parasite control
Effective vaccination programme (e.g., leptospirosis, bovine viral diarrhoea, infectious bovine rhinotracheitis)
Premating
Ensure adequate live weight at the time of mating (at least 65% of mature body weight)
Maintain a high plane of nutrition around mating; vital that weight is gained rather than lost
Mating to calving
Ensure adequate growth rates to achieve at least 85% of mature body weight at first calving (Fig. 22.33)
Reduce growth rate in the last 2 months to reduce the risk of dystocia



• **Fig. 22.33** Live weight (line graph) and daily liveweight gain (bars) of heifers. (Adapted from Drew 2004.)

upsurge of interest in the relationship between nutrition and fertility, especially in regard to the marked changes that have occurred in the metabolic endocrine system of dairy cows as a result of genetic selection for increased lactation performance.

Of course, nutrition has always been recognised a key determinant of reproductive performance, not only in dairy cattle but also

TABLE 22.9 Effect of live weight of Holstein Heifers at first calving on subsequent in-calf rates (at 6 and 21 weeks) and conception rates during their first lactation

Precalving Live Weight (kg)	N	6-Week in-Calf Rate (%)	21-Week in-Calf Rate (%)	Proportion (%) Conceiving 7–21 Weeks After the Start of Mating
≤ 400	319	49	79	30
401–440	388	60	87	27
441–470	369	68	89	21
471–510	450	68	87	19
511–540	226	75	88	13
≥ 541	231	77	87	10

With permission from Morton (2000b).

in beef animals. The increase in energy demands that is associated with the onset of lactation is not immediately matched by an increase in feed intake, and there is a lag of several weeks between peak lactation and peak feed intake. Hence early lactation is usually characterised by a period of negative energy balance when tissue is mobilised to meet the demands for lactation that are not being supplied through feed. Negative energy balance is at its worst 1 to 2 weeks after calving, but it can persist well into lactation (Dillon et al. 2003), beyond the period of maximum yield (Hattan et al. 2001). In high yielding cows, appetite may not be able to satisfy energy requirements, even when using energy-dense diets, until yields have started to decline (Beever et al. 2001), well into the period when it would be appropriate to start serving cows. For high yielding cows that have a standard 305-day lactation, precalving body condition may not have been reached by the time of drying off (Beever et al. 2004).

Animals have a clear prioritisation for energy when in negative energy balance, such that maintenance and lactation take precedence over growth and reproduction: i.e., when negative energy balance exists, there is a consequential impairment of reproductive performance (Bauman & Currie 1980). Cows that are in negative energy balance during early lactation are at greater risk of becoming anoestrous than those that are in energy equilibrium (Zulu et al. 2002a). Likewise, cows that are in negative energy balance are more likely to remain in anoestrus until equilibrium is restored (Butler & Smith 1989), which is probably the reason that the persistence of lactation in Holstein cows is negatively associated with reproductive performance (Buckley et al. 2003). Conception rates and embryonic mortality rates are also adversely affected in animals that are in negative energy balance (Gossen et al. 2006, Grimard et al. 2006, Roche et al. 2007).

Maintaining body condition score (BCS) in cows that have been genetically selected for high yield is particularly challenging, especially in systems that are forage- or pasture-based. Roche et al. (2006) found that North American Holstein cows fed pasture plus concentrates lost approximately 65 kg live weight (compared with ~35 kg in New Zealand Friesian cattle) over the first 2 months of lactation. Holsteins receiving 6 kg of concentrates per day did not achieve precalving body weight until more than 200 days into lactation, whereas those on lower levels of concentrates had not

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TABLE 22.10 Body condition score at calving, postcalving condition score loss and pregnancy rates

Criterion	Pregnant Within 6 Weeks of the Start of Mating (%)
Body Condition Score at Calving (1 - 8)	
< 4.5	58
4.5–5.4	69
> 5.4	65
Body Condition Change During Early Lactation (1 to 8 Scale)	
Lost ≥ 0.67 units	58
Lost 0.33–0.67 units	59
Lost < 0.33 units	61

From Morton (2000b).

returned to precalving body weights by 300 days into lactation. It is interesting to note that, when BCS rather than live weight is assessed, Holsteins require an even longer period of time to return to precalving values (Roche et al. 2006), probably because changes in gut fill with advancing lactation contribute substantially to changes in live weight (Beever et al. 2004). The net result of these changes in live weight and BCS is that significantly more Holstein than Friesian cows (30% vs 3%; Kolver et al. 2006) failed to conceive by the end of the mating period. Fahey et al. (2005) also found that Holstein cows fed pasture plus limited concentrate supplementation had a longer anoestrous period than adequately fed animals. Taken together, these studies show that both excessive BCS loss (Chapa et al. 2001, Gossen et al. 2006) and the depth of the BCS nadir (Buckley et al. 2003, Roche et al. 2007) appear to be the main determinants of the degree to which reproductive performance (e.g., the duration of anoestrus, first-service conception rate, calving-to-conception interval) is impaired.

There is, however, a complex relationship between the effects of BCS at calving and energy balance during the postcalving period. Reasonable body stores of fat at the time of calving can provide a store of energy that can mitigate the effects of undernutrition. Thus whereas BCS loss in the postcalving period is negatively associated with the duration of anoestrus and conception rates (Roche et al. 2007), optimal BCS at calving is associated with better reproductive performance than low BCS (Patton et al. 2007). The InCalf survey of Australian dairy cattle (Morton 2000b) clearly illustrates this pattern (Table 22.10). Conversely, calving in too high a condition score also adversely affects reproductive performance. Cows that are fat at calving (Ohgi et al. 2005, Gossen et al. 2006) or have been fed excessively during the precalving period (McNamara et al. 2003) have higher live weight loss due to lower postcalving feed intake (Butler 2005). Consequentially, such animals have increases in NEFAs and β-hydroxybutyrate (βOHB), decreases in glucose, and increases in indicators of hepatic dysfunction due to lipid infiltration of the liver (Zulu et al. 2002a). There are strongly negative relationships between elevated blood NEFA/βOHB concentrations and reproductive outcomes, particularly in relation to ovarian follicular development (Marr et al. 2002) and oocyte competence (McEvoy et al. 1997). As a result, conception rates and pregnancy rates are likely to be significantly depressed; Samarutel et al. (2006), for example, found that no cows with a BSC of greater than 3.75 (1–5 scale) conceived to their first insemination. On the other hand, significant loss of live weight over the dry period is also associated with metabolic disorders and impaired reproductive performance in the postcalving period (Ill-Hwa & Gook-Hyun 2003).

Thus reasonable body stores of fat at the time of calving can provide a store of energy that can mitigate the effects of undernutrition, whereas excessive mobilisation of fat from overweight animals is undesirable, especially if there is a significant degree of underfeeding postcalving. However, if it is possible that energy demands of the freshly calved cow can be substantially met, calving with only a moderate condition score can be preferable to calving with excessive stores of fat. Hence, within each farming system, practices have been empirically developed that best approximate the type and amount of feed that is available for the nutrient requirements of lactation in order to minimise the duration/incidence of anoestrus. In traditional British dairying systems the preferred option has been for cows to calve in a moderately fat condition, because the grass silages and cereals available to British dairy farmers were of inadequate quality to fully feed freshly calved cows. More recent British practice has followed that of North America, in which the dairy cows hold relatively small reserves of body fat but whose appetite is capacious and, when given high quality forages and energy-dense cereals, can approximately match output by intake. Pastoral dairying cannot fully feed cows in the early postcalving period, so tries to find an equilibrium point of calving condition score that allows enough fat reserves to meet the energy deficit while not being so fat that appetite is limited. Farm advisors, nutritionists, and veterinarians who operate within these systems become adept at optimally balancing these factors in order to both maximise lactation yields and minimise postcalving anoestrus.

Nutrition and Reproduction in the Beef Suckler Cow

Similar relationships between BSC loss and fertility occur in beef cows. Losing weight is associated with poor fertility, whereas gaining weight is more likely to be associated with better fertility. Any postcalving loss of more than 5% is likely to be a cause for concern (Morris 1976). In a meta-analysis of the responses of beef suckler cows to oestrus synchronisation, Stevenson et al. (2015) found that parous cows with BCS of 5 or higher (1 (worst) to 9 (best) scale) had the highest conception rates and the lowest levels of embryonic loss, whereas primiparous cows with BCS lower than 5 performed the most poorly for these traits. Soca et al. (2014a) also found substantially poorer reproductive performance in beef cows that were in low BCS (<3.5; 1–8 scale) at calving than in those in moderate BCS (≥4); the postpartum anoestrous interval was 119 versus 94 days, and the probability of an early pregnancy was 0.46 versus 0.84.

These effects of BCS are additive with the effects of suckling. Suckling inhibits the release (but not synthesis) of GnRH, resulting in suppressed follicular development particularly in the early postpartum period. The mechanism for this suppression has long been held to be a prolactin-mediated inhibition of the reproductive neuroendocrine axis, but the literature review by Yavas and Walton (2000) suggested that this is not the case. Rather, they suggested that oxytocin and endogenous endorphins are likely mediators, although there are concurrent effects upon IGF-1, which may underpin the suckling–nutrition interaction that occurs in beef suckler cows. Suckling restriction over the start of the breeding period, even without complete calf removal, improves reproductive performance (Quintans et al. 2010). Even those suckler cows that do ovulate when being suckled are prone to irregular oestrous cycles, which may be due to oxytocin-mediated premature luteolysis

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of corpora lutea (Martin et al. 2010). On the other hand, restriction of suckling has been reported to increase concentrations of IGF-1 (Soca et al. 2014b), which may promote the development of follicles to the preovulatory stage.

Energy Metabolism and Reproduction

The last few years have seen the recognition that the fertility of dairy cows has been declining (McGowan et al. 1996; O'Farrell et al. 1997; Dillon & Buckley. 1998). This decline was initially attributed to the many changes in the husbandry and management of dairy cows that have occurred over the past couple of decades, but more recently it has been recognised that the intensive selection for high milk yield that has taken place (particularly in the Holstein–Friesian breed) has been associated with a parallel, unintentional, selection for worsening fertility.

Genetics and Heritability

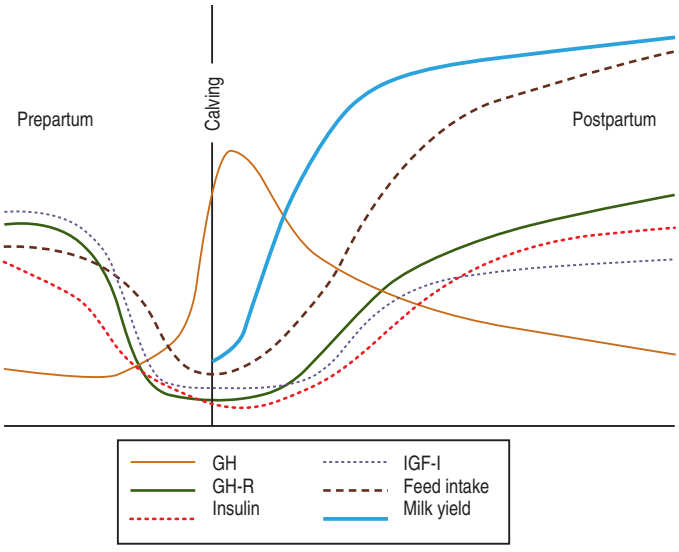
The traditional view has been that the heritability of fertility traits is very low and the magnitude of any genetic correlations between production and fertility traits so marginal that any genetic effect of selection for high yield upon fertility traits would be negligible (Raheja et al. 1989, Weller 1989, Mantysaari & van Vleck 1989, Arendonk et al. 1989). More recently, this opinion has changed. Many studies (e.g., Boichard & Manfredi 1994, Hoekstra et al. 1994, Dematawewa & Berger 1998) recorded genetic relationships between fertility and production traits in US Holsteins that were high and negative. Even in the Swiss Simmental, often regarded as a dual purpose rather than a dairy breed, a negative genetic association was found between yield and fertility (Hodel et al. 1995). As a result, it has now become widely accepted that there is an inverse genetic relationship between yield and fertility that is manifest most strongly in the North American Holstein and animals derived from it. Flint (2006) even refers to infertility as an 'inherited disease' of cattle. Heritability of binary traits such as conception to first service remains relatively low but, by linking yield to fertility through continuous variables such as interval to first luteal activity (Royal et al. 2002), embryonic mortality (Grimard et al. 2006), dairy conformation (Royal et al. 2002, Berry et al. 2004, Dechow et al. 2004), and proportion of Holstein genetics (Wall et al. 2005), more useful heritabilities have been identified. Indices based on BCS have also been explored: some studies have not considered them valuable (e.g., Banos et al. 2004) whereas others have come to the opposite conclusion (e.g., Royal et al. 2002, Berry et al. 2004).

In consequence, there has been a progressive trend to incorporate fertility and longevity traits into selection indices (Mantysaari & van Vleck 1989, O'Farrell 1998, Burton & Harris 1999), as a means of preventing a further decline in fertility or even of reversing the trend. Most studies of the relationship between fertility and yield have recorded a cohort of cows that have both high yield and acceptable fertility: identification and selection of such individuals is advocated as a means of improving fertility without compromising yield (e.g., Kadarmideen & Wegmann 2003). The use of genomic, rather than phenotypic, selection processes for dairy cows appears to be having some success in this, not least because it circumvents some of the problems caused by inability to directly assess fertility traits versus the direct assessment of production. A major thrust of selection for fertility has therefore been centred on the use of quantitative trait loci (QTLs) associated with a female fertility index (FTI), which is a composite of various parameters of fertility (Hoglund et al. 2015), or which are associated with functional longevity (Punsmann & Distl 2017). Use of QTL technology has also allowed the identification of genetic parameters associated with individual parameters of reproductive performance (Carthy et al. 2015), some of which have proved to be closely identified with physiological parameters such as steroidogenesis (Ortega et al. 2016). García-Ruiz et al. (2016) considered that, in the 7 years since genomic selection was introduced for US Holsteins, selection has improved for low heritability fertility characteristics such as daughter pregnancy rate and productive life.

Energy Metabolism

Cows that have been genetically selected for high yield have a number of differences in the activity of their metabolic endocrine system from cows with a lower genetic potential, which appear to be of importance in the mechanism of their subfertility. Growth hormone (GH) is the chief hormone responsible for nutrient partition in dairy cows. Cows that are genetically selected for high yield have higher blood GH concentrations than lower-yielding and beef cattle, whereas administration of bST (i.e., GH) is a pharmacological stimulus to greater lactational yield.

Growth hormone acts through receptors in liver and adipose tissue to regulate the supply of glucose and fatty acids to the mammary gland (Lucy 2006). The liver also produces IGF-I in response to GH, with a negative feedback loop between hepatic IGF-I and hypothalamic GH releasing hormone (GHRH) (and hence pituitary GH) secretion. Liver GH receptors (GHR) decrease abruptly just before calving, resulting in reduced IGF-I secretion (Lucy et al. 2001, Zulu et al. 2002b). Loss of the negative feedback regulation of GH by IGF-I means that its concentrations rise (Fig. 22.34), resulting in gluconeogenesis in the liver and lipolysis in adipose tissue. Hence there is a net decrease in IGF-I concentrations and a net rise in concentrations of NEFA and other products of lipolysis in the postpartum period. Insulin appears to be a key mediator in this process (Fig. 22.35). High yielding cows have lower insulin concentrations and a much greater degree of peripheral insulin resistance than lower yielding cows (Wathes et al. 2007), and the massive drain on blood glucose into the mammary gland means that insulin concentrations are maintained at low values. Low insulin concentrations, in turn, continue to suppress hepatic



• **Fig. 22.34** Sequence of changes in concentrations of key metabolic hormones and metabolites during the pericalving period and the onset of lactation. (Adapted from Lucy et al. 2001.)

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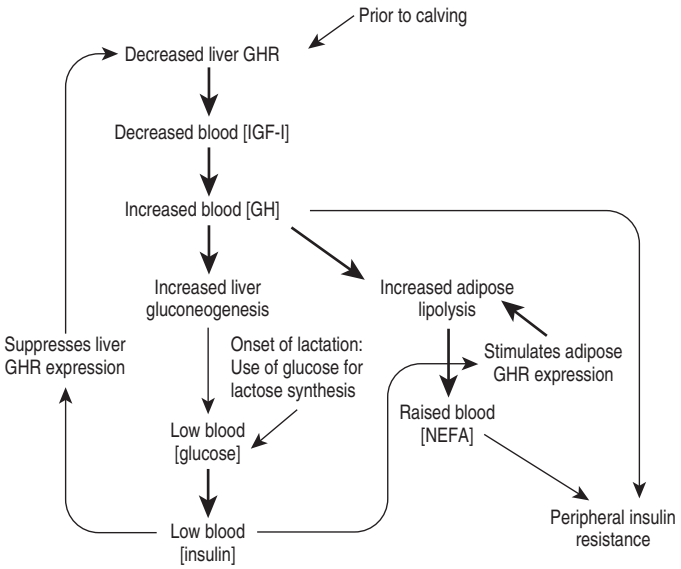
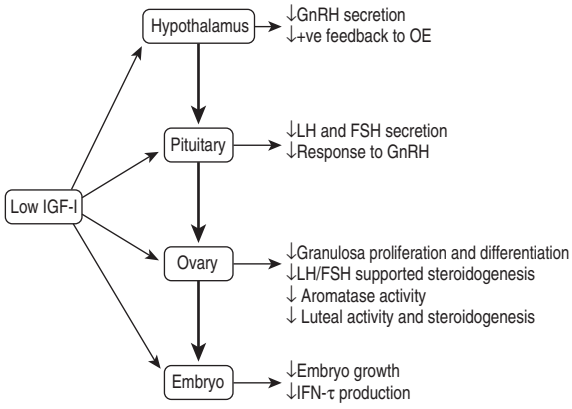


• **Fig. 22.35** Interrelationships between GH, IGF-I, insulin, and negative energy balance in the postpartum, high yielding dairy cow. (Based on data in Lucy 2006.)



• **Fig. 22.36** Effects of low circulating IGF-I concentrations on the reproductive system. (Adapted from Zulu et al. 2002a, b, and Butler 2005.)

GHR expression, resulting in the maintenance of the catabolic state. Once glucose supply starts to exceed demand, the situation becomes reversed.

Strategies to increase glucose supply include modifying rumen fermentation to increase the supply of propionate or to directly supply gluconeogenic substrates (Patton et al. 2004). Thus feeding monensin (Hixon et al. 1982), monopropylene glycol (Tunon et al. 2006), or increasing the starch component of the diet have all been associated with improved reproductive outcomes.

In the underfed cow (Lucy et al. 2001, Wang et al. 2003), there is also a decline in liver GHR and IGF-I concentrations, leading to sustained GH secretions and the consequent stimulation of lipolysis. The effects of impaired or altered energy nutrition upon reproduction are therefore largely the result of the interaction of low concentrations of IGF-I (Fig. 22.36), insulin, and glucose and high concentrations of NEFA and βOHB with the reproductive endocrine axis.

Low blood glucose and elevated βOHB and NEFA concentrations result in impaired GnRH and/or LH secretion (Butler & Smith 1989, Beam & Butler 1999) with consequent effects upon follicle growth and ovulation. This is particularly noticeable in cows with fatty liver, in which basal, pulsatile, and preovulatory LH are abnormal (Reid 1984). Second, both IGF-I and insulin are important growth factors for the growth and development of follicles (Simpson et al. 1994, Armstrong et al. 2002, Zulu et al. 2002a). The actions of IGF-1 are primarily mediated through granulosa cell proliferation (Wathes et al. 2003) and enhanced responsiveness to gonadotrophins (Zulu et al. 2002a), whereas insulin primarily affects oestrogen production (Wathes et al. 2003). There is also some stimulation of aromatase activity by IGF-I. Low IGF-I concentrations are therefore associated with underdevelopment of follicles in underfed cows (Webb et al. 1997), whereas cows with short postpartum anoestrous intervals (Thatcher et al. 1996) and normal ovarian function (Zulu et al. 2002b) have higher IGF-I concentrations than do those with delayed or abnormal ovarian activity. Third, there is evidence that negative energy balance has deleterious effects upon the oocyte (Kruip et al. 1998, Butler 2005), primarily due to the effects of raised NEFA concentrations (Kruip et al. 2001). These effects are not limited to the duration of elevated NEFA concentrations, however, and may persist well into lactation (Snijders et al. 2000). Even when insulin concentrations are elevated through concentrate feeding, residual adverse effects upon the oocyte persist (Garnsworthy 2006).

Steroidogenesis in the CL is impaired in the absence of adequate concentrations of insulin or IGF-I (Liebermann et al. 1996). Conversely, luteal cells have receptors for GH (Wathes et al. 2003), so the relationships between high yield, negative energy balance, and progesterone synthesis are relatively complex. On the other hand, progesterone concentrations in high yielding cows are reduced by its increased metabolism in the liver (Lucy & Crooker 2001, Sangsritavong et al. 2002). Thus regardless of whether the cause is impaired luteal synthesis or enhanced metabolism, high yielding cows and animals in negative energy balance generally have lower circulating progesterone concentrations during the luteal phase or early pregnancy than other animals. Low progesterone concentrations are associated with a reduced probability of pregnancy, at least partly due to retarded embryo development and a consequent inadequate production of IFN-τ to prevent luteolysis (Mann et al. 2006).

Protein

High yielding dairy cows require around 18% to 19% crude protein (CP) to sustain lactation (Kung & Huber 1983). Degradable protein and non-protein nitrogen sources that are incorporated into microbial protein are able to maintain moderate yield, but, in order to meet the nutrient demands for high yields, sources of undegradable protein are also needed. In terms of maintaining normal reproductive function, however, high protein levels are not needed, with a number of studies suggesting that approximately 16% is adequate for the maintenance of reproductive activity (Jordan & Swanson 1979, Ferguson & Chalupa 1989). Protein undernutrition (<16% CP) has, however, been associated with impaired reproductive function in some (Jordan & Swanson 1979, Fekete et al. 1996) but not all (Edwards et al. 1980) studies. One mechanism by which protein deficiency may affect reproductive activity is via a decrease in GH receptor expression in the liver (Breier 1999) and a reduction in IGF-I release in response to exogenous GH (McGuire et al. 1992, Lucy et al. 2001).

The effect of high levels of dietary protein upon conception rate has been the subject of long-standing controversy: many studies show an adverse effect of high protein levels upon fertility, whereas many others show no effect (Lean et al. 1998).

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The potential for high levels of protein to affect fertility has to be considered in terms of the overall metabolic effects of excess protein. These effects are well recognised:

- When energy supply is limiting and the rate of ammonia production from dietary CP exceeds the ability of the rumen microbes to convert it into microbial protein, excess nitrogen will be absorbed as ammonia (Wathes et al. 2003). This is particularly a feature of diets that are deficient in readily fermentable carbohydrates and have a significant excess of degradable protein (e.g., spring pasture).
- Excess ammonia has to be converted to urea by the liver. This has two consequences. First, there is an energy cost associated with the synthesis of urea, which can be an additional drain on the energy resources of an underfed cow (Roche 2006). Second, blood urea concentrations rise, with potentially negative consequences on a number of aspects of reproductive activity.

The energy cost of metabolising the ammonia that is liberated from excess protein is generally accepted to be such that excess degradable protein may exacerbate negative energy balance during early lactation and thereby reduce fertility (Butler 1998). There are a number of possible routes by which urea might affect reproduction:

- Abnormally high concentrations of urea and ammonia may be present in the uterus (Jordan et al. 1983), which may be toxic to spermatozoa (Jordan & Swanson 1979), oocytes, or embryos (Ferguson 1990, Elrod & Butler 1993, Robinson & McEvoy 1996), or adversely affect uterine function (Elrod 1992, Butler 1998, Rhoads et al. 2004).
- High circulating concentrations of urea may also have an effect upon the hypothalamic–pituitary axis, particularly LH (Jordan & Swanson 1979, Blauwiekel et al. 1986).
- There may be an impairment of luteal progesterone synthesis, resulting in reduced circulating concentrations (Larson et al. 1997), although others (Ferguson & Chalupa 1989) have found no effect of high CP inclusions on progesterone concentrations.
- There may be adverse effects of urea upon hepatic clearance rates of reproductive steroids (Lean et al. 1998).
- High dietary CP may be associated with an increased incidence of retained fetal membranes, dystocia, and postpartum metritis (Anderson & Barton, 1987).

Meta-analyses of field trials (Gerloff & Morrow 1986, Lean et al. 1998) concluded that a marginal depression of conception rate occurs at CP levels of 16% to 18% and that the reduction is significant at higher than 19% CP. Laven et al. (2007), on the other hand, came to the conclusion that cattle can tolerate high nitrogen intakes without their fertility being adversely affected, probably because of their ability to adapt to high blood urea concentrations (Dawuda et al. 2002). They concluded that the effect of excess dietary nitrogen upon reproduction is primarily a function of the interval between the timing of the increase in nitrogen intake relative to fertilisation or ovulation (i.e., with a shorter interval increasing the likelihood of an effect) rather than to CP or rumen degradable protein (RDP) contents per se. Hence the effects of excess nitrogen are more likely to be related to acute increases in blood ammonia (and hence follicle, oocyte, and intrauterine ammonia) in the preadaptation period than to those of urea (which represent a postadaptation situation). However, when excess nitrogen causes a significant drain on the energy resources of cows that are already in negative energy balance, this may impair reproduction via energy-mediated pathways.

Interestingly, a number of studies have shown that small improvements in the UDP content of the diet improves fertility, or at least mitigates the effects of high levels of RDP (Armstrong et al. 1990, Staples et al. 1998a). This may, as suggested by McClure (1994) and Webb et al. (1997) improve the availability of gluconeogenic substrates. However, the most common source of high grade, undegraded protein for such trials is fish meal, a feedstuff that also contains unique fatty acids that can affect not only carbohydrate metabolism but also prostaglandin and steroid metabolism (Staples et al. 1998b, Meier 2000, Verkerk 2000), so the effects are not necessarily a direct effect of UDP.

Minerals and Micronutrients

Effects Upon Fertility

Although it is generally agreed that micronutrients can affect fertility of cattle, there are conflicting opinions about the significance of apparent deficiencies. This is because of the inherent difficulties of accurately determining nutrient requirements, because of lack of knowledge of the interaction of micronutrients in the alimentary tract, and because many of the studies that determined nutrient requirements were done 40 or more years ago when yields were much lower. McClure (1994) also pointed out that many older studies attributed beneficial effects of micronutrient supplementation upon fertility to comparisons of reproductive performance before and after supplementations, a technique that is now considered to be invalid. Most micronutrient deficiencies exert their effects upon reproduction through (i) depression of the activity of rumen microflora, (ii) reduction in enzyme activity affecting energy and protein metabolism and the synthesis of hormones, (iii) integrity of rapidly dividing cells within the reproductive system, and (iv) the role of micronutrients as antioxidants, which are responsible for protecting cells from the effects of free radicals.

Cobalt

Cobalt deficiency occurs in pastures of Australia, New Zealand, Florida, Kenya, and Scotland (McClure 1994). Deficiency occurs when diets contain less than 0.07 mg/kg dry matter (DM) cobalt and is due to the failure of vitamin B_{12} synthesis, which is an essential cofactor for carbohydrate metabolism. Deficiency usually causes anaemia, inappetence, poor bodily condition, ill thrift, and loss of condition. Poor fertility (non-display of oestrus, irregular interoestrus intervals, and poor pregnancy rates) may be present at the same time as these obvious signs of deficiency. Sometimes poor fertility in apparently normal cows can be corrected by cobalt supplementation.

Diagnosis can be confirmed by measuring liver cobalt or vitamin B_{12} concentrations. The concentration of cobalt in the liver of cattle is sufficiently responsive to changes in cobalt intake to make it a useful aid in the diagnosis of cobalt deficiency in the field. Similarly, the responsiveness of vitamin B_{12} concentrations to suboptimal cobalt intakes makes it a useful diagnostic aid. Blood concentrations of cobalt or vitamin B_{12} can also be used.

The mode of action of vitamin B_{12} in carbohydrate metabolism is well-recognised, but it is less clear whether it has direct effects upon the reproductive system. Gagnon et al. (2015) showed that vitamin B_{12} supplementation was associated with the activation of a range of genes in dominant follicles, resulting in larger follicles than in untreated animals.

Copper and Molybdenum

Hypocuprosis can be either direct or indirect. Direct copper deficiency, due to an absolute deficiency of copper in the diet,

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TABLE 22.11 Diagnosis of copper deficiency: lower limits of 'marginal' bands[a]

Criterion[b]	Diet	Sheep and Cattle[c]	Interpretive Limits
Diet Cu:Mo	H	**1.0**–3.0	Diet S >2g kg^{-1} DM
	F	**0.5**–2.0	Diet Mo <15 mg kg^{-1} DM for cattle
Diet Fe:Cu	H	50–**100**	
Diet Cu (mg kg^{-1} DM)	H	**6**–8	Diet Mo <1.5 mg kg^{-1} DM
	F	**4**–6	
Liver Cu (µmol kg^{-1} DM)[d,e]	H or F	**100**–300	
Plasma Cu (µmol l^{-1})[e]	H or F	**3**–9	Diet Mo <15mg kg^{-1} DM for cattle: no acute phase response; age >1 week
Blood Cu (µmol l^{-1})	H or F	**6**–10	
Hair or wool Cu (mg kg^{-1} DM)	H or F	**2**–4 (sheep)	Clean sample, recent growth
		4–8 (cattle)	

[a]Means within marginal band indicate a probability of CU-RD that increases with proximity to the **bold** limit, while values beyond the band do not. As the *number of* individuals falling within a marginal band increases, so does the likelihood of heard or flock benefit from Cu supplementation.
[b]Alternative criteria such caesruloplasmin and erythrocyte CuZnSOD activities are not included because they introduce additional problems of interpretation or standardization without adding significantly to the precision of diagnosis.
[c]Some data for cervines from Wilson and Grace (2001).
[d]Divide by 3.0 to obtain values on fresh weight basis.
[e]Multiply by 0.064 to obtain values in mg.

is exceedingly uncommon (Suttle 2010). Most cases of copper deficiency are indirect, as a result of excessive levels of molybdenum, iron, or sulphur (and, possibly, calcium or zinc) in the diet, which result in the conversion of copper into an insoluble, indigestible form within the rumen (Phillippo 1983, McClure 1994, Underwood & Suttle 1999). Hypocuprosis is most likely when there is high soil molybdenum, high soil pH (e.g., after liming; Phillippo 1983), immature plants, excess rainfall/irrigation, and/or contamination of forage with soil (Suttle 2004). Decreased use of sulphur-containing fertilisers and avoidance of excess levels of iron inclusion in mineral feed supplements appears to be associated with a decrease in the prevalence of indirect copper deficiency in the UK over recent years (Suttle 2010). It has been suggested that excess dietary molybdenum may have direct effects that mimic many of the features of hypocuprosis (Phillippo et al. 1982), although Suttle (2010) discounts this possibility. The main consequence of copper deficiency is upon the efficiency of food utilisation because of effects upon the integrity of the small intestine and of loss of activity of energy–metabolism (McClure 1994). Copper also has a role as an antioxidant, which may be of importance to reproductive performance (Lean et al. 1998).

Copper deficiency has long been associated with impaired fertility. This has been manifested as a reduction in first-service conception rates, impaired embryonic survival, a reduction in overall pregnancy rates, and poor display of oestrus. Impaired LH secretion has been implicated as the main means by which these effects may be mediated (Phillippo et al. 1982, 1987). Effects can range from negligible to pronounced (Corah 1996). All of the effects of hypocuprosis on fertility appear to depend on a concurrent excess of dietary molybdenum (Phillippo et al. 1982, 1987). Some studies have suggested that fertility may be responsive to copper supplementation (e.g., Pickering 1975), but other studies have suggested that fertility is not related to blood copper concentrations (e.g., Larson et al. 1980) nor does copper supplementation necessarily have a positive effect on fertility (Whittaker 1980). Moreover, in a number of studies in which copper deficiency has clearly been documented, there often is no effect on fertility or reproductive performance (e.g., Corah 1996). Indeed, Hawkins (2014) found an adverse effect of injecting CaCu EDTA 10 days before the start of mating upon subsequent reproductive performance.

One of the problems with determining whether copper deficiency is affecting fertility is finding an accurate measurement of copper status (Laven & Livesey 2005) because neither blood nor liver copper concentrations nor blood ceruloplasmin concentrations provide a full representation of animals' copper status. Suttle (2010) suggested the use of multiple criteria and, if there are a number of these that are below the lower limit of the 'marginal' band, a diagnosis of hypocuprosis is likely (Table 22.11). Values above the cutoff indicate that there is little likelihood of copper supplementation being beneficial.

Iodine and Goitrogens

Simple iodine deficiency occurs when intakes are less than 0.8 mg/kg DM (Alderman 1970) to 2.0 mg/kg DM (McClure 1994). Lean et al. (1998) considered that percutaneous absorption of iodine-based teat dips is adequate to prevent deficiency in milking cows. Iodine deficiency is largely manifested through the effects of a lack of thyroxine. As thyroxine is a general metabolic regulator (McDonald & Pineda 1989), its deficiency is associated with non-specific signs of ill thrift and poor growth. The main effects of iodine deficiency upon reproduction are also related to impaired thyroid function in the dam, embryo, or fetus. The most common abnormalities associated with iodine deficiency are embryonic death, abortion, and stillborn or weak, goitrous calves. Loss of libido and inhibition of oestrus behaviour (although not necessarily of ovarian cycles) have also been reported (Spielman et al. 1945, Williams & Stott 1966, McDonald 1980). However, the results of iodine supplementation trials (e.g., with injection of iodised oil; Logan et al. 1991, Mee 1991) upon fertility traits other than stillbirths are equivocal (Anderson et al. 2007).

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Disturbance of thyroid function can also be due to goitrogenic substance present in kale, lentils, soya bean, linseed, and certain strains of white clover (Boyd & Reid 1961, Tassell 1967). High levels of such goitrogenic substances can produce anoestrus in heifers (David 1965).

Manganese

Manganese is a cofactor in a number of enzymes that are responsible for gluconeogenesis (McClure 1994) and has a significant role as an antioxidant (Lean et al. 1998). Manganese is also involved in cholesterol synthesis and hence steroidogenesis. Both the pituitary gland and ovaries are relatively rich in this trace element. A variety of reproductive disorders that depress fertility in cows have been blamed on manganese deficiency, including delayed puberty, anoestrus, poor follicular development, delayed ovulation, silent oestrus, and reduced conception rates (Lean 1987, Hurley & Doane 1989, Kendall & Bone 2006). It also causes joint and limb deformities in calves. Under normal circumstances it is likely that normal pasture will provide the necessary requirement of 80 mg/kg DM (Alderman & Stranks 1967), although some foods (e.g., maize silage) are low in manganese. In addition, there is an interaction with the calcium to phosphorus ratio in the diet, with some evidence that high liming of pasture can cause manganese deficiency.

Phosphorus

The evidence for the importance of hypophosphataemia as a cause of infertility is conflicting. The provision of supplementary phosphorus has been shown to improve the breeding performance of grazing cattle (Sheehy 1946, Hart & Mitchell 1965, Tassell 1967). A number of authors have described infertility that was characterised by anoestrus, suboestrus, irregular cycles, and low conception rates (Hignett & Hignett 1951, Morrow 1969, Morris 1976), in the absence of other clinical signs of phosphorus deficiency. Others (Littlejohn & Lewis 1960, Cohen 1975, Carstairs et al. 1980) found no evidence of reduced fertility associated with phosphorus deficiency.

Daily phosphorus intakes should be about 13 g/day for maintenance, with about 7 g extra for each 4.5 litres of milk (Deas et al. 1979). Most forages contain adequate levels to meet these demands but deficiencies may occur on phosphate-deficient soils (McClure 1975). Morris (1976) suggested that deficiency can occur when the phosphorus content of the feed is under approximately 0.25%. High yielding cows need phosphorus in excess of that available in pasture, but because cereal grains contain large amounts, deficiencies are unlikely to occur. Blood phosphorus concentrations of less than 4 mg/dL in animals at peak production are indicative of deficiency. If hypophosphataemia is suspected, a rapid response can follow the feeding of dicalcium phosphate (150–200 g/day) or bone meal.

Selenium and Vitamin E

One of the key roles of selenium is as a component of the cytosolic enzyme glutathione peroxidase (GPX). Cytosolic GPX acts in concert with other antioxidants to prevent damage to cellular membranes induced by free radicals that arise from cellular metabolism of oxygen (Wichtel 1998a). However, many other selenium-containing enzymes exist, including iodothyronine deiodases and other glutathione peroxidases, some of which have functions that are quite distinct from the antioxidant role of cytosolic GPX. The roles of selenium are therefore widespread throughout the body:

- Lipid peroxidation: Selenium, in concert with the active derivative of vitamin E, α-tocopherol, prevents lipid peroxidation of membranes, particularly of the skeletal and cardiac muscles.
- Arachidonic rachidonic acid metabolism: Glutathione peroxidases are involved in the metabolism of arachidonic acid into the intermediate prostaglandins PGG and PGH (Hong et al. 1989).
- Phagocyte activity: Selenium deficiency, mediated through glutathione peroxidases, is associated with reduced bacteriocidal activity of neutrophils, reduced proliferation of lymphocytes and increased release of peroxides and macrophages (Wichtel 1998a).
- Iodothyronine 5-deiodinase: The enzyme, which converts thyroxine into the active thyroid hormone, triiodothyronine, is selenium-containing (Berry et al. 1991). Hence the growth retardation that is associated with selenium deficiency appears to be due, at least in part, to impaired thyroid function.

A wide range of effects of selenium deficiency on reproductive performance have been reported, including retained fetal membranes, poor uterine involution, metritis, cystic ovaries, and impaired fertilisation (Trinder et al. 1973, Harrison et al. 1986). Wichtel (1998a) noted that the effects of selenium deficiency on uterine involution and postpartum ovarian activity could be mediated through its effects on immune function, uterine contractility, thyroid hormone activity, or prostaglandin synthesis (Segerson et al. 1980, Hong et al. 1989, Spallholz et al. 1990, Wichtel et al. 1996).

Selenium deficiency can occur when soils contain <0.5 mg/kg or the diet contains <0.05 mg/kg selenium. Vitamin E deficiency occurs when animals graze postmature pasture, receive other dietary components that contain <0.7 mg/kg of the vitamin, or are fed diets that are high in polyunsaturated or rancid fats (McClure 1994). Diagnosis of selenium deficiency can be made by measuring circulating concentrations of selenium or, better, by measuring selenium stores in the liver (Table 22.12; Wichtel 1998b). Measurement of levels in feed, pasture, or soil is also often indicated. Supplementation is widely practised, especially in areas in which soils are known to be marginal or deficient. However, it should be remembered that excessive selenium is toxic, especially when it has been given by injection.

TABLE 22.12 Selenium reference ranges related to the growth of young cattle

	Whole Blood Se (nmol/l)	Serum Se (nmol/l)	Glutathione Peroxidase (kU/l)	Liver Se (nmol/kg)
Responsive	< 130	< 52	< 0.5	< 380
Marginal	130–250	52–100	0.5–2.0	380–650
Adequate	> 250	> 100	> 2.0	> 650

From Wichtel (1998b).

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Studies involving selenium-deficient Friesian–Holstein heifers have shown improved pregnancy rates after treatment (MacPherson et al. 1987). Taylor et al. (1979) found a lower abortion rate after selenium supplementation, whilst McClure et al. (1986) reported a higher first-service conception rate after selenium supplementation. Vitamin E deficiency is directly associated with embryonic loss in cows and, through its role in the immune system, may also affect the rate of uterine involution after calving (Lean et al. 1998).

Vitamin A and β-carotene

Diets that are deficient in β-carotene can result in impairment through direct effects of β-carotene and through vitamin A deficiency. McClure (1994) suggested that deficiency only occurs when animals have been on diets that contain less than 45 mg/kg β-carotene for a considerable period of time.

Early studies (Kuhlman & Gallup 1942, Madsen & Davis 1949, Byers et al. 1956) showed that vitamin A deficiency is associated with delayed puberty, low pregnancy rates, and poor conception rates. Subsequently, Lotthammer (1979) identified a direct effect of β-carotene though studies on feeds that contained adequate levels of vitamin A but were deficient in β-carotene. Diets that are deficient in β-carotene, but are adequate in vitamin A, have been reported to result in extended follicular phases, delayed ovulation, silent oestrus, follicular cysts (Lotthammer et al. 1978), low conception rates, low pregnancy rates (Cooke 1978), and impaired luteal progesterone synthesis (Jackson 1981).

However, not all studies have agreed with these results. Bindas et al. (1983), Ducker et al. (1984), Marcek et al. (1985), and Gossen and Hoedemaker (2005) all found that reproductive performance was unresponsive to β-carotene. The reasons for the different responses are difficult to explain. The current view of β-carotene is that, if it has a role in reproduction, it is probably related to its activity as an antioxidant, perhaps protecting the maturation of the oocyte (Ikeda et al. 2005) or development of the early embryo.

Zinc

Zinc is actively involved in a range of enzyme functions. The necessity for zinc appears to be greater in males (Hurley & Doane 1989) than in females (Pitts et al. 1966). Zinc deficiency in the bull may cause impaired fertility associated with an alteration in the latter stages of spermatogenesis (Corah 1996). This impairment of male infertility appears to be associated with the role of zinc as an activator of enzymes involved in testosterone synthesis. Zinc deficiency in the female may cause a decrease in fertility and abnormal oestrus behaviour (Corah & Ives 1991).

The uptake of zinc is impaired by copper, calcium, iron, molybdenum, and cadmium. However, Suttle (1994) suggested that the presence of zinc in soil and in galvanised pipeware means that deficiency is unlikely to occur. Whether there is any benefit from chelated zinc supplements is therefore open to question (Underwood & Suttle 1999). Zinc toxicity is rare, but excessive zinc supplementation may lead to perturbation of essential fatty acid metabolism and hence affect prostaglandin synthesis.

Antioxidant Function

A number of micronutrients are believed to act upon reproductive performance primarily through their role as antioxidants. Many metabolic processes produce superoxides that, through the Fenton reaction, produce highly destructive free radicals (Fettman 1991). Normally, the tissue-damaging effects of these free radicals are prevented by the presence of antioxidants. Many of these are essential micronutrients; so, when these micronutrients become deficient, free radical tissue damage occurs (Lean et al. 1998). Free radical damage includes the creation of toxic lipids, reactive proteins, free radical cascades, and nucleic acid damage. Transition metals, by virtue of their ability to change oxidation states, are the key component of many antioxidant systems.

McClure (1994) listed selenium, α-tocopherol, β-carotene, and copper as the key antioxidants. To this list, Lean et al. (1998) added manganese, zinc, iron, cobalt, and vitamin A. Glucose, sulphur-containing amino acids, and various proteins probably also exert some antioxidant role. Dietary sources, active forms, sites of action, and mechanism of action of the major antioxidants are listed in Table 22.13.

Phyto-oestrogens

When cows ingest large quantities of these substances they become anoestrous, with large ovarian cysts, vulval and cervical enlargement, and poor conception rates (Morris 1976). Such substances are found in subterranean clover, certain strains of red and white clover, and lucerne.

Investigation of Nutritional Factors as a Cause of Infertility

It is frequently impossible to accurately determine a specific nutritional cause of infertility because the clinical signs appear sometime after the deficiency has occurred. Methods such as the use of cumulative frequency graphs of conception rate or monitoring daily bulk milk protein concentrations can help to pinpoint the times when management changes took place that have adversely affected nutrition (see Chapter 25). Effects of improving nutrition are more difficult to determine because changes in the season of the year and management can also have an effect that can obfuscate the effects of changes in diet.

In most cases, the most important factor responsible for poor fertility is underfeeding. This is most commonly due to:

- Overestimation of the feeding value of forages. For conserved forage, it is important to obtain accurate analysis of the major food components from truly representative samples. For pasture, it is important to accurately estimate both the amount and the digestibility of the material that is present;
- Overestimation of feed intake under self-feed conditions. Self-fed silage is especially susceptible to overestimation. However, it should be noted that, in most situations in which self-feeding is practised, especially when there is inadequate feeding space for all cows to feed simultaneously, vulnerable cows (i.e., first calvers, smaller cows, etc.) will be at a significant disadvantage, in terms of feed intake, than their herd mates;
- Failure to appreciate the reduction of forage intake caused by high concentrate intake (Alderman 1970); and
- Underfeeding of concentrates due to automatic dispensers giving short measure. Parker and Blowey (1976) found errors of more than 50% in some cases. Manual dispensers in the milking shed are more commonly inaccurate than accurate.

It is necessary to calculate the requirements of the cows for maintenance and production and then obtain accurate information about the precise quantities fed. Contributions from mineral licks and other free access sources are difficult to quantify. Weighing, the use of a girth band measure, or condition scoring of a representative number of animals are also useful.

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TABLE 22.13 Dietary sources, active forms, sites of action and mechanism of action of the major antioxidants

Dietary Input	Biologically Active Antioxidant	Site of Action	Mechanism of Action
Selenium	Glutathione peroxidase	IC, membrane	Reduces peroxides
Copper	Cu/Zn superoxide dismutase	IC	Scavenges O_2^-
	Ceruloplasmin	EC	Binds Cu, oxidises Fe
	Superoxide dismutase	EC	Scavenges O_2^-
Zinc	Cu/Zn superoxide dismutase	IC	Scavenges O_2^-
	Superoxide dismutase	EC	Scavenges O_2^-
	Metallothionein	EC	Binds metal ions
Manganese	Mn superoxide dismutase	IC	Scavenges O_2^-
Iron	Catalase	IC	Reduces peroxides
	Transferrin	ED	Binds iron
Cobalt	Vitamin B12		
Vitamin E	α–tocopherol	Membrane	Blocks peroxidation
Vitamin A	Retinol	EC	Maintains cell integrity
β-carotene	β-carotene	Membrane	Singlet oxygen
	Retinol	EC	Maintains cell integrity
Glucose	Ascorbate	EC	Radical scavenger
Sulphur-containing amino acids	Glutathione	IC	Replenishes glutathione peroxidase
Protein	Various	EC	Binds metal ions, scavenges OH

IC, intracellular; *EC,* extracellular.
With permission from Lean et al. (1998).

Metabolic Profiles

Metabolic profiles were introduced in 1970 (Payne et al. 1970). Since then, they have frequently been used to help in the evaluation of the nutritional status of a herd, particularly in relation to fertility. Metabolic profiles are best used in conjunction with other direct assessments of nutrient quality (such as ME, digestibility, RDP, and UDP estimates of forages) and supply, together with milk volume and fat/protein content (Whittaker 2004). Samples that are commonly collected for interpretation of energy status are glucose, NEFA, and βOHB; for protein, samples include urea, albumin, and globulin (Table 22.14).

Other samples that are sometimes incorporated into metabolic profiles include the following:

- **Bile acids:** Although included as a means of assessing lipid infiltration of the liver, this is not reliable; liver biopsy is preferable.
- **Calcium:** As homoeostatic control is strong, calcium measurements are of little value except in clinical cases.
- **Magnesium:** Values below the normal range (0.8–1.3 mmol/L) may be indicative of incipient grass staggers if very low or of impairment of production due to subclinical hypomagnesaemia if marginal.
- **Inorganic phosphate:** Phosphorus deficiency is not common, so phosphate status should be checked before adding supplementary phosphate to the diet.
- **Copper:** Blood copper concentrations are not a reliable indicator of copper status (require liver samples), although concentrations decline when liver copper stores are depleted.
- **Glutathione peroxidase:** Because GPX concentrations are a long-term rather than short-term measure of selenium status, care is needed in interpreting results.
- **Liver enzymes** were initially commonly incorporated into metabolic profiles to provide an indicator of liver health. It is probably not cost effective to do so unless there is a specific indication for assessment of the liver.

Details of the tests and their evaluation are available in standard texts of cattle medicine.

Other Factors Affecting Reproductive Performance

Heat Stress

The temperature zone of comfort for dairy cows is between 5°C and 25°C (McDowell et al. 1979) and, when the environmental temperature rises above 25°C, reproductive performance is impaired. The effects of high temperature are exacerbated under conditions of high humidity (Gwazdauskas et al. 1973); calculation of a temperature–humidity index (THI) is a useful predictor of the degree of thermal stress to which cows are subject. The consequence of a high THI is that there is a significant reduction in conception rate in the hot season compared with the winter season; literature reviewed by De Rensis and Scaramuzzi (2003) showed a reduction of conception rate of 20% to 30% in

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TABLE 22.14 Normal ranges and interpretation of samples collected for metabolic profiles

Sample	Optimal Range (Lactating Cows)	Interpretation	Advantages	Disadvantages
Energy				
β-hydroxybutyrate	< 1.0 mmol/L	0.6–1.0 mmol/L: acceptable level of fat mobilisation > 2.0 mmol/L: ketosis	Stable in transit	Butyrate may be absorbed from butyric silage
Non-esterified fatty acids	< 0.7 mmol/L	> 1.5 mmol/L: ketosis	More direct measure of fat mobilisation than βOHB	Less stable in samples than βOHB May be raised in stressed animals
Glucose	> 3.0 mmol/L (plasma)	Variation within optimum range according to weight change Very high values in stressed animals	Sensitive to short-term changes in fermentable ME	Close homoeostatic regulation Must use oxalate-fluoride anticoagulant Measure in plasma rather than serum or whole blood
Protein				
Urea	Urea N: > 1.7 mmol/L Urea N: > 3.3 mmol/L Urea: > 3.6 mmol/L	Low: inadequate RDP:FME ratio Inadequate FME in diet High: excess RDP:FME		
Albumin	> 30 g/L	Low: poor liver health long-term underfeeding of protein		Serum samples have lower values than plasma
Globulin	< 50 g/L	High: indicates presence of infection		Not related to how acute or severe a disease process is

Derived from Whittaker (1997, 2004).

the hot season. Adverse effects upon reproductive performance can be particularly significant for high producing dairy cows in tropical/subtropical environments, even to the point of making dairying unsustainable under some conditions. Moreover, the effects of heat stress do not end as soon as the temperature cools to below the upper threshold, because there is good evidence of the effects of heat stress persisting into the autumn (De Rensis et al. 2017).

The main effects of heat stress upon reproduction in female cattle are upon follicular growth, ovulation, the expression of oestrus (Schuller et al. 2017), and upon the establishment of pregnancy (Fig. 22.37). Oestrus expression is reduced in heat stressed cows, in terms of the duration of oestrus and the ability of herd managers to detect oestrus (Hansen 2007). This is partly due to decreased locomotor activity (De Rensis et al. 2015). Up to 80% of oestrus periods may be undetected (Thatcher & Collier 1986) during periods of heat stress. It is also partly due to impaired development of preovulatory follicles. Heat stressed cows have abnormalities of dominant follicle hierarchy (Roth et al. 2000), resulting in lower oestradiol concentrations (Thatcher & Collier 1986, Lu et al. 1992) than in unstressed animals. Impaired follicle development, in turn, occurs due to impaired GnRH secretion and, thence, impaired LH and FSH secretion. The cause of the reduced activity of the reproductive endocrine axis is not fully understood but has been explained in terms of the effects of elevated prolactin or cortisol concentrations, inappetence, and impaired acid–base balance in heat stressed animals (De Rensis et al. 2017). In consequence, there is an increased prevalence of ovulation failure (López-Gatius et al. 2005), twin, and other abnormal ovulations, and there is impairment of oocyte development (Zeron et al. 2001) and maturation (Pavani et al. 2017). There is also an increased risk of either failure of luteal formation or the formation of CLs whose progesterone secretion is inadequate.

Many studies have shown that the establishment of pregnancy is also impaired when ambient temperatures are high (e.g., Barker et al. 1994). The effects of heat stress are primarily upon the early embryo. Most studies have shown that fertilisation rates are normal (Thatcher & Collier 1986, Wise et al. 1988), but embryonic death occurs between fertilisation and maternal recognition of pregnancy. Most deaths occur during the early cleavage divisions (Roman-Ponce et al. 1981, Putney et al. 1989, Ealy et al. 1993), although a lower rate of deaths also occurs between Days 8 and 16 (Biggers et al. 1986). Hansen (2007) suggested that the effects of heat stress are mediated through damage to the oocyte, impaired protein synthesis during the morula and blastocyst phases and, possibly, through impaired endometrial activity.

Effects of heat stress are also seen in later gestation. Significant pregnancy loss continues up to Day 50 of gestation (López-Gatius et al. 2004). Furthermore, placenta weight and calf birth weight are reduced in cows that calved in hot months versus those calving in cooler months or versus cows provided with cooling during the hot months (Thatcher & Collier 1986, Wolfenson et al. 1988). Thus heat stress may not only affect pregnancy rates if it occurs at the time of fertilisation but also impair pregnancy throughout its course.

The effects of heat stress can be overcome to a greater or lesser extent by cooling cows by providing shade (Vermeulen 1988), water sprays (Omar et al. 1996), or sprays used in combination with forced ventilation (Flamenbaum et al. 1988, Lu et al. 1992). Alternatively, or additionally, hormone treatments and fixed time AI can be used to circumvent the effects of heat stress. De Rensis et al. (2015) noted that hormone treatments to manage the effects

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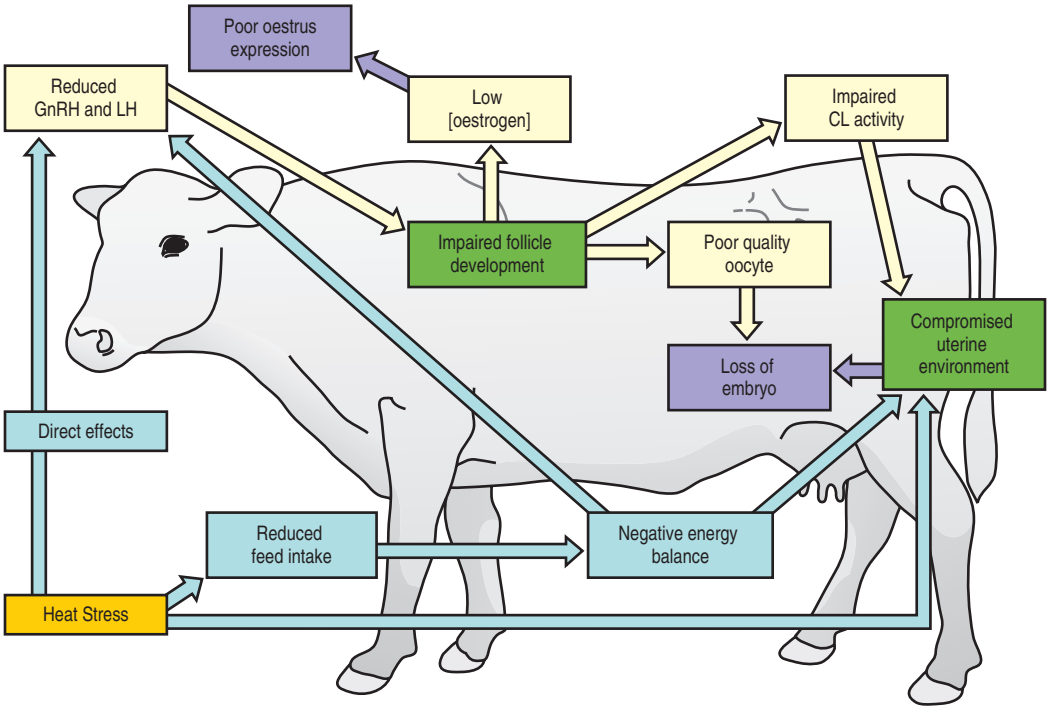

• **Fig. 22.37** Possible mechanisms for the effect of heat stress on reproduction in the lactating dairy cow. Heat stress can act directly through the **reproductive endocrine system**, through **feed intake,** and directly upon the uterus. In consequence, **follicle development** is impaired and early embryo losses increase, resulting in infertility. The poor follicle development also causes a reduction in **oestrus expression**. (Based upon De Rensis & Scaramuzzi 2003.)

of heat stress should be suitable for fixe time AI, resolve anoestrus and abnormalities of ovulation, and be of short duration. GnRH given at the time of insemination increases conception rate (Ullah et al. 1996), but the response to Ovsynch (GnRH–$PGF_{2\alpha}$–GnRH) is variable and not particularly beneficial. However, replacement of GnRH with hCG or eCG appears to be beneficial in cows that are heat stressed, and administration of hCG after AI may also improve pregnancy rates (De Rensis et al. 2008, Shabankareh et al. 2010). Similarly, inclusion of progesterone in the synchronisation protocol may improve conception rates (Garcia-Ispierto et al. 2013). On the other hand, administration of progesterone after insemination does not appear to have any significant effect upon pregnancy outcomes (e.g., Correa-Calderon et al. 2016).

Hence, although heat stress causes a significant impairment of many parameters of reproductive performance (anoestrus, lowered conception rates, reduced calf birth weight, and, possibly, impaired postpartum uterine involution), simple management procedures are generally sufficient to mitigate or alleviate the problem. Simply providing shade and adequate access to water helps considerably, and cooling with water and/or forced ventilation is highly effective. In the longer term, selection for heat tolerance may be feasible; there are clear differences between breeds in terms of heat tolerance, and there are a number of candidate genes that have significant genetic variability within breeds (e.g., Macciotta et al. 2017).

The 'Repeat Breeder' Syndrome

The term 'repeat breeder' was coined to describe cows that failed to conceive after a number of services and regularly return to oestrus at a normal inter-oestrus interval. By mathematical chance, if cows have a 60% conception rate, about 6.4% of the animals in a herd will not have conceived after the third mating. It was originally considered that chance was the only factor in determining whether these animals failed to conceive. However, when the cows that repeatedly fail to conceive have been examined in more detail, it has been clear that they are not the random group of animals that mathematical probability would suggest, but rather contain a subset of cattle that are actually subfertile. Moreover, Barrett et al. (2004) showed that, rather than approximately 6% of animals being non-pregnant after the third service, the figure is closer to 12%, the difference between the theoretical and actual figures also being composed of a group of subfertile animals.

Early work on repeat breeders was responsible for the identification and subsequent elimination of some of the major venereal pathogens of cattle; yet, even when this had been achieved, there remained an irreducible proportion of cows that experienced repeated pregnancy failure. Some repeat breeders are cows that simply have gross pathology of the reproductive system, some functional form of infertility, or experience some managemental predisposition to infertility. A number of reviews have enumerated the causes of reproductive failure in such animals (Roberts 1986, Lafi & Kaneene 1988, Eddy 1994, Levine 1999). Many of these causes have been considered earlier in the chapter and can be diagnosed by careful clinical examination and history taking. The repeat breeder cow that presents the greatest clinical and managemental challenge, however, is the animal that continually returns to service in the absence of any obvious pathological disease.

Impaired Embryonic Development

Ayalon and co-workers undertook a number of pivotal studies of the repeat breeder cow. They found a slightly reduced fertilisation rate in repeat breeders compared with normal cows and that, after fertilisation, the two groups of cows exhibited very different patterns of embryonic survival. Repeat breeders suffered a significant level

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TABLE 22.15 Embryonic death in normal and repeat-breeder cows

Day After Fertilisation	PERCENTAGE OF ANIMALS WITH EMBRYOS	
	Normal	Repeat Breeder
2–3	85	71
11–13	74	50
14–16	73	50
17–19	60	43
35–42	67	35

Compiled from Sreenan & Diskin (1986; normal cows) and Ayalon (1978; repeat breeders).

of embryonic death (Ayalon 1972, 1978, Maurer & Echternkamp 1985) at around Day 6 pregnancy, with further losses at around Days 17 to 19 (Table 22.15). These timings are associated, first, with hatching from the zona pellucida and, second, with failure of the maternal recognition of pregnancy on Day 16. Embryos derived from normal cows failed to survive in the uteri of repeat breeders, whereas embryos derived from repeat breeders had normal survival rates in normal cows (Almedia et al. 1984, Ayalon 1984). Furthermore, the fertilisation and cleavage rates of oocytes collected by ovum pick-up and fertilised *in vitro* are similar in repeat breeder and normal cows (Bage 2003). Hence the problem of the repeat breeder is primarily in the uterine environment rather than representing a deficiency of the embryo itself, although by the seventh day of gestation, the *in vitro* developmental capacity of embryos derived from repeat breeders is compromised (Tanabe et al. 1985), as is their morphological development (Gustafsson 1985).

Thus provided one has excluded obvious pathological lesions, mismanagement of mating, and infectious diseases that impair reproductive performance, the three main causes of repeat breeding remain namely, abnormalities of the periovulatory period, chronic endometrial damage, and luteal deficiency.

Periovulatory Abnormalities

A number of studies of repeat breeder cows have shown endocrine abnormalities of the periovulatory period. Repeat breeders are likely to have delayed ovulation, an extended follicular phase and reduced signs of oestrus (Sood et al. 2015). These abnormalities appear to be associated with the presence of suprabasal progesterone concentrations during the follicular phase (Bage 2003, Bhupender et al. 2005), which Vos (1999) suggested allows follicle development but postpones the LH surge. Hence there is a longer interval between luteolysis and ovulation and a delayed LH surge, resulting in follicles and oocytes that are relatively aged by the time of ovulation. Repeat breeder cows are more likely to have two- than three-wave cycles (Perez et al. 2003), further increasing the probability of the presence of aged follicles. Repeat breeders are also more likely to have a delayed postovulatory rise in progesterone concentrations (Bage et al. 2002), possibly as a result of the impairment of the events around ovulation.

Luteal Deficiency

Progesterone is necessary for the maintenance of pregnancy. Until Day 150 to 200 of pregnancy the main source of the hormone is the CL, so if it is not completely formed or it is not functioning adequately, then insufficient progesterone is produced, and the pregnancy is at risk of failure. Luteal deficiency has been suspected of causing infertility for many years, and, although proof is difficult, treatment of repeat breeders is frequently based on this assumption.

The relationship between progesterone concentrations and pregnancy rate has been investigated on many occasions. Most of these studies agree that blood or milk progesterone concentrations in pregnant animals diverge from those that fail to conceive at some point between Days 6 and 14 postinsemination (e.g., Bulman & Lamming 1978, Lamming et al. 1989) and that this divergence is likely to be of importance for the success of embryo development. Kendall et al. (2009) also found that low milk progesterone concentrations on Day 5 (<3 ng/mL) was more common in repeat breeders (34%) than in normal cows (11%). Hence Starbuck et al. (1999) considered that pregnancy is unlikely if milk progesterone concentrations are less than 3 ng/mL on Day 5 post-insemination. One of the key effects of progesterone is upon trophoblast expansion: low progesterone concentrations are associated with suboptimal growth of the blastocyst and hence insufficient secretion of IFN-τ to prevent luteolysis (Demmers et al. 2001).

Damage to the Endometrium

Although chronic endometritis has been associated with the repeat breeding syndrome at an epidemiological level (e.g., Moss et al. 2002), some doubt has been cast on the importance of uterine infection. For example, Levine (1999) did not place great importance upon chronic uterine infection as a cause of repeat breeding, citing studies by Hartigan et al. (1972), DeKruif (1976), Hartigan (1978), and Roberts (1986) as evidence of a generally low infection rate and moderate bacterial recovery rates. More recently, this view was confirmed by Pothmann et al. (2015), who found evidence of subclinical endometritis in only 12.7% of repeat breeders; and by Jaureguiberry et al. (2017) who diagnosed subclinical endometritis (≥ 5% neutrophils in cervical cytobrush samples: see Chapter 23) in 24% of repeat breeders. The latter study also found that the presence of fluid in the uterine lumen was of better diagnostic value for repeat breeders than were cytobrush samples. Interestingly, studies from the Indian subcontinent do not support this view because, for example, Ramakrishna (1996) found 46 out of 60 repeat breeder cows to have significant bacterial isolates from cervical discharges, and Malik et al. (1987) found 370 out of 396 mucus samples from infertile cows to be infected. Similarly, Ahmadi et al. (2017) reported bacterial biofilms in the uterus of 12 out of 20 repeat breeder cows.

Moreover, infection does not have to be active at the time of sampling for infection-related uterine damage to have occurred. The effects of infection on endometrial scarring in the mare are well known (see Chapter 31), but even in the cow, there is increasingly clear evidence that chronic uterine damage results from infection (see Chapter 23). Gonzalez (1984) associated infertility with the degree of endometrial damage that was present in uterine biopsies, whilst DeBois and Manspeaker (1986), in their review of endometrial biopsies in cattle, noted that mild chronic endometritis is one of the most common causes of repeat breeding. Hence, it was their opinion that endometrial biopsy is an essential part of the examination of the valuable cow with unexplained infertility.

Uterine secretions of repeat breeder cows have been characterised as differing from those of normal cows (Zavy & Giesert 1994). Almedia et al. (1984) showed qualitative and quantitative differences in the ionic composition of uterine flushings of normal and repeat breeder cows. No clear-cut associations between biopsy lesions and uterine secretion characteristics are evident in the literature, and it might be argued that differences in uterine secretory

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profiles are as likely to reflect differences in luteal activity (or, at least, progesterone status) as the degree of histological change to endometrial structure. On the other hand, more recent genetic studies of repeat breeders have associated increases in interleukins and chemokine ligand in the lymphocytes in those with chronic endometritis, dysregulating of mucin genes in animals with and without chronic endometritis (Wagener et al. 2017), as well as abnormalities of TNF-α (Kalyaan et al. 2015).

Treatment

Given the relationship between low progesterone and pregnancy failure, attempts have been made to find cost-effective ways to augment circulating progesterone concentrations in the hope of improving the pregnancy rate in an entire herd, especially among the repeat breeders.

The main luteotrophic hormone of the cow is LH. Thus, if LH activity is enhanced (e.g., by injection of hCG or GnRH) after ovulation, stimulation of the development and function of the CL may result. Despite the long-standing use of hCG as a 'holding injection' in infertile cows, there is no statistically significant effect in improving pregnancy rates in either normal cows (Greve & Lehn-Jensen 1982, Sreenan & Diskin 1983) or repeat breeders (Hansel et al. 1976, Leidl et al. 1979).

Results for the use of GnRH at the time of insemination have generally been positive (Schels and Mostafawi 1978, Lee et al. 1981, Nakao et al. 1983, Morgan & Lean 1993). Hence the use of GnRH in this way is quite common (Malmo & Beggs 2000). Lean et al. (2003) undertook a meta-analysis of 40 trials on the administration of GnRH at insemination, with the result that the overall risk of pregnancy was increased by 12.5% in all cows but was increased by 22.5% when used in repeat breeders.

An alternative approach has been to give hCG or GnRH 11 to 13 days after breeding. The rationale for this approach is to induce accessory corpora lutea or luteinised follicles, or to augment the progesterone secretion from existing structures. Macmillan et al. (1986) and Sheldon and Dobson (1993) recorded benefits from such a regimen, but not all studies agree with this (e.g., Jubb et al. 1990). Meta-analysis of the effects of 8 to 10 μg buserelin or 250 μg GnRH on Days 11 to 14 after insemination (Lean et al. 2003) found that the risk of pregnancy to first service was increased by 7% to 10% at the first service but not at subsequent services. Nonetheless, they concluded that treatment with GnRH is likely to be cost effective.

Progesterone implants have been used to try to augment progesterone concentrations during the period when luteolysis is expected. However, Diskin and Sreenan (1986) concluded from meta-analysis of many early trials of the effects of progesterone administration upon pregnancy rate that it was ineffectual. However, interest in progesterone administration as a means of augmenting pregnancy rate has been rekindled, and a method that is increasingly widely practised is the reinsertion of previously used progesterone-releasing intravaginal devices (i.e., PRIDs or CIDRs). This practice may increase pregnancy rates (Macmillan et al. 1986) by augmenting circulating progesterone concentrations, but it has an additional advantage that returns to oestrus in non-pregnant cows are either synchronised or occur at a predictable time (Cavalieri et al. 2000a, b).

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23

The Metritis Complex in Cattle

I. MARTIN SHELDON

Introduction

Microbial infections of the female genital tract often cause disease in cattle after calving. Postpartum microbial disease of the female genital tract is particularly common in *Bos taurus* dairy cattle, whereas disease is less common in beef breeds of *Bos taurus* and in *Bos indicus* cattle. Uterine disease is typified by the accumulation of pus in the genital tract, which often discharges from the vulva (Fig. 23.1).

Uterine disease is important in cattle because the consequences are subfertility and, in some cases, infertility. For example, bacterial infections of the uterus causing metritis lead to delayed insemination, reduced conception rates, and increased culling for failure to conceive. The cost to the dairy industry for treating metritis, reduced milk production, and replacing animals that fail to conceive at the farmer's desired time is estimated to be €1.4 billion every year in the EU (Sheldon et al. 2009). This chapter sets out the definitions, causes, diagnosis, treatment, and management of postpartum, non-specific uterine infectious diseases in cattle.

Defining the Diseases of the Postpartum Uterus

The definitions and brief details of non-specific, postpartum infectious diseases are presented first to provide a framework for understanding the aetiology, pathogenesis, diagnosis, and treatment of uterine disease. The typical incidence and duration of uterine disease is shown in Fig. 23.2.

Retained Fetal Membranes

Retained fetal membranes (RFM, see also Chapter 20) are defined as the failure of an animal to expel the fetal membranes within 24 hours of the end of parturition. Retained placenta is an alternative name used for RFM. There is some variation in the literature about the duration of retention that defines the clinical disorder. Some prefer to define retention as being for 12 hours; however, the timing is arbitrary, and most normal cows expel the fetal membranes within a few hours of parturition. The incidence of RFM varies among herds, but is typically 5% to 10% of animals. The importance of RFM is that they are associated with reduced milk yield and an increased risk of metritis.

Metritis

Metritis is most common within 10 days of parturition. Metritis is characterised by an enlarged uterus and a watery red-brown fluid to viscous off-white purulent, uterine discharge, which often has a fetid odour (Sheldon et al. 2006). The incidence of metritis varies between breed, country, and herd, but in a study of the records from 97,318 cows in the US, the lactation incidence of metritis, including RFM, was 21% (Zwald et al. 2004). However, in some studies the incidence is as high as 40% of the herd. The associated clinical signs are used to classify the severity of disease, which varies from unapparent disease to fatal toxaemic metritis.

Clinical Endometritis

Clinical endometritis is defined as the presence of a purulent uterine discharge detectable in the vagina of cattle 21 days or more postpartum or a mucopurulent discharge detectable in the vagina after 26 days postpartum, (Sheldon et al. 2006). The incidence of clinical endometritis is around 10% to 20%, with variation between breed, country, and herd; a typical study reported that 16.9% of 1865 dairy cows were affected in Canada (LeBlanc et al. 2002a). A simple grading system based on the character of the vaginal mucus is readily used to evaluate cows with clinical endometritis and is prognostic for the likely outcome of treatment (Sheldon et al. 2006).

Subclinical Endometritis

Subclinical endometritis is characterised by inflammation of the endometrium in the absence of clinical signs of endometritis, which results in a significant reduction in reproductive performance. The inflammation is presumably associated with recovery of the tissues after metritis and clinical endometritis, trauma, or other non-microbial disease. Definition of subclinical disease is currently dependent on cytological analysis of samples that are collected from the surface of the endometrium by flushing the uterine lumen or by the use of an endometrial cytobrush. The proportion of animals affected varies widely among studies, ranging from about 11% to more than 40% of animals (Wagener et al. 2017). Diagnosis relies on the proportion of endometrial cells that contain more than a defined proportion of neutrophils at specific times after parturition.

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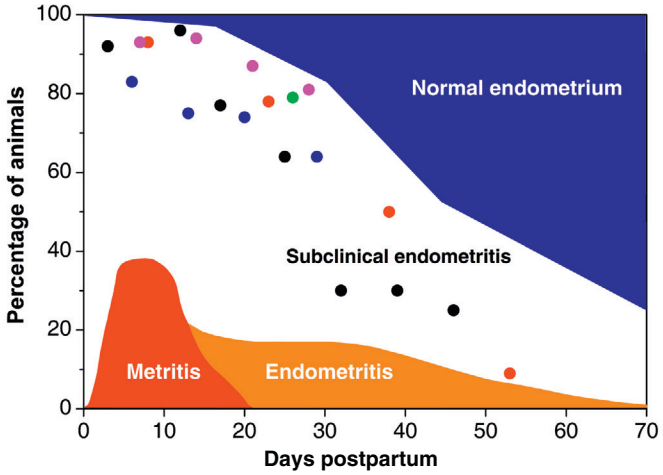
• **Fig. 23.1** A typical case of postpartum metritis in a dairy cow with accumulation of pus in the uterus, which is discharging from the vulva.



• **Fig. 23.2** The incidence of uterine bacterial infection and disease in postpartum dairy cattle (From Sheldon et al. 2009). Bacteria can be isolated from the uterus of most cows during the postpartum period; each marker (*circle*) indicates the percentage of animals with bacteria isolated from the uterine lumen in four studies (Elliott et al. 1968, Griffin et al. 1974b, Bonnett et al. 1991a, Sheldon et al. 2002b). The shaded areas represent estimates of the proportion of animals with metritis (*red*), clinical endometritis (*orange*), or a normal uterus (*blue*); the remainder of animals have subclinical endometritis (Gilbert et al. 2005).

Pyometra

Pyometra is characterised by the accumulation of purulent or mucopurulent material within the uterine lumen, causing distension of the uterus, in the presence of a closed cervix and a functional corpus luteum. Postpartum pyometra is uncommon and is thought to be caused by the growth of bacteria within the uterine lumen after the formation of the first corpus luteum (Noakes et al. 1990).

Inflammation in Other Parts of the Reproductive System

Oophoritis, cervicitis, and vaginitis are defined as inflammation of the oviduct, cervix, and vagina, respectively. The signs are typical of the cardinal signs of inflammation: redness, heat, swelling, pain, and loss of function. Although the incidence of oophoritis is often unclear, cervicitis and vaginitis are relatively common.

The Postpartum Period

The main reproductive events that are required after parturition to foster optimal future fertility are: prompt expulsion of the fetal membranes, followed by uterine involution; timely return of ovarian cyclical activity; regeneration of the endometrium; and efficient control of bacteria in the uterus.

Expulsion of the Fetal Membranes

The primary mechanism by which the caruncle and cotyledon of the placentome anchor to each other is the interdigitating fetal and maternal villi, and the presence of adhesive proteins at the fetomaternal interface. The surrounding and engulfing of the caruncle by the cotyledon provides physical anchoring (Eiler & Fecteau 2007). Detachment of the membranes requires release of the cotyledon from the caruncle by disruption of the physical and cellular arrangements that are present during pregnancy. There appear to be three main factors involved in the separation and expulsion of the fetal membranes:

- Maturation of the placenta, including biochemical and immunological changes at the fetal–maternal junction;
- Exsanguination of the fetal side of the placenta when the umbilicus ruptures, which causes collapse and shrinkage of the trophectodermal villi and their physical separation from the maternal crypts; and
- Uterine contractions, which aid in the exsanguination of the fetal side of the placenta and aid in the physical separation of the placenta by distorting the shape of the placentomes, thereby causing 'unbuttoning' of the cotyledon from the caruncle, and attempting to expel the dependent and detached parts of the fetal membranes.

Preparatory changes for the release of the fetal membranes are not confined to the peripartum period but begin during the late stage of gestation (Grunert 1986). These changes appear to be largely dependent on a critical sequence of changes in the concentrations of progesterone and oestrogen and the abundance of their cognate steroid receptors (Agthe & Kolm 1975, Chew et al. 1977, Boos et al. 2000). For example, the presence of subnormal oestrogen and oestrogen receptor concentrations may lead to RFM.

Physical changes that occur in association with the release of the fetal membranes include flattening of the maternal crypt epithelium and, in the last week of gestation, a reduction in the number of binucleate cells in the trophectoderm (Bjorkman 1969, Gross & Williams 1988). Relaxation of the superficial layers of the cotyledon is induced by proteases and collagenase, which break down the type I and III collagen that provides the tensile strength to the caruncular crypts (Boos et al. 2003, Eiler & Fecteau 2007). Likewise, collagenases also disrupt the anchoring attachments between the fetal and maternal villi. Failure of proteolysis is considered to be a key factor in failure of membrane detachment, although, curiously, there is no difference in the distribution of types I, III, or IV collagen in cows that do or do not retain their membranes (Sarges et al. 1998, Boos et al. 2003). Inhibition of collagenase activity by exogenous glucocorticoids appears to be a major contributing factor to the retention of the membranes associated with the premature induction of parturition with glucocorticoids.

Changes in the composition of the glue line adhesive proteins, which are present between the cotyledonary and caruncular epithelium, are of importance in the process of placental detachment.

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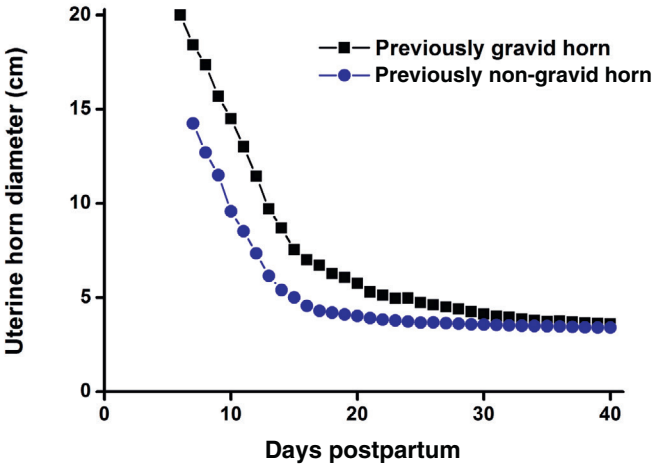
Proteoglycans are intercellular adhesion proteins that are present at the fetomaternal interface: there is evidence that the activity of the enzyme beta-*N*-acetyl-glucosaminidase, which is responsible for the metabolism of proteoglycans, is reduced in cows with RFM (Kankofer et al. 2000).

Changes in synthesis and metabolism of prostaglandins E and F are also associated with the process of detachment. Circulating concentrations of PGFM (13, 14-dihydro, 15-keto $PGF_{2\alpha}$, a stable metabolite of $PGF_{2\alpha}$) are higher and those of PGEM (a metabolite of PGE2) are lower in normal cows than in those with RFM (Wischral et al. 2001b). Placentomes from cows with RFM produce less $PGF_{2\alpha}$ and more PGE2 *in vitro* than do those from normal cows (Gross et al. 1987), and there is also interconversion of PGF to PGE in cows with RFM (Kankofer et al. 1994). Moreover, PGE secretion continues after parturition, as the retained membranes retain a degree of viability for some days (Eiler & Fecteau 2007). The PGF secretion in placental tissue from normal cows is responsive to stimulation by oxytocin, whereas that from animals with RFM is not (Slama et al. 1994). Deficiency of PGF synthesis is likely to be the consequence of a lack of maternal oestrogen synthesis, resulting in a failure of accumulation of prostaglandin precursors and arachidonic and linoleic acids in placental tissues (Wischral et al. 2001a, Wischral et al. 2001b).

Failure of oestrogen synthesis in the placenta is associated with inadequate placental or fetal antioxidant activity, in terms of peroxide damage to lipids, protein, and nucleic acids (Wischral et al. 2001b, Kankofer & Guz 2003, Gupta et al. 2005). It is probably for this reason that dietary deficiencies of antioxidants such as selenium and vitamin E predispose to RFM (Bourne et al. 2007). Excess PGE synthesis may be a response to maternal stress and oxidative injury or a response to infection (Wischral et al. 2001b, Herath et al. 2009a). As PGE is a potent immunosuppressive agent, PGE secretion may adversely affect the immunological environment of the postpartum uterus.

There is increasing evidence that the immune system plays a pivotal role in detachment of the membranes (Heuwieser & Grunert 1987, Peters & Laven 1996). Neutrophil, macrophage, and T lymphocyte invasion of the placenta occurs around the time of detachment, and there is evidence that deficiencies in the activities of each of these cells occurs in cows with RFM (Gunnink 1984, Kimura et al. 2002, Miyoshi et al. 2002). Cows with RFM also produce reduced quantities of the neutrophil attractant interleukin-8 (IL-8, also known as CXCL8) and other inflammatory mediators (Slama et al. 1993, Miyoshi et al. 2002, Davies et al. 2004). Moreover, in cows with RFM the activity of the neutrophil antiendotoxin protein, acyloxyacyl hydrolase, is reduced (Dosogne et al. 1999). This may put cows at greater risks from the effects of the endotoxin from bacteria that proliferate in the uterus postpartum. Finally, as leucocytes contribute to the collagenase activity of the parturient uterus, reduced leucocyte activity in cows with RFM may be a contributor to the reduction in collagen proteolysis in these animals.

The role of the cells of the immune system may be due in part to the role of MHC class I antigens in the process of placental detachment. It has been postulated that maternal recognition of fetal MHC antigens provides an initial trigger for placental separation because MHC class I compatibility between a cow and her fetus has been associated with an increased incidence of placental retention (Davies et al. 2004, Eiler & Fecteau 2007). It has been suggested that this indicates that alloreactivity to the fetal membranes by the dam is a significant event in the detachment of the placenta (Joosten et al. 1991).



• **Fig. 23.3** Uterine involution monitored in 24 dairy cattle, using transrectal ultrasonography to measure the diameter of the previously gravid and non-gravid uterine horns at the level of the attachment of the intercornual ligament (From Sheldon et al. 2003).

Uterine Involution

Involution is the term used to describe the physical reduction in size of the uterus and cervix after parturition (also see Chapter 7). Involution is thought to be driven by uterine muscular contractions, turnover of the extracellular matrix, necrosis and sloughing of the uterine caruncles, and regeneration of the endometrium (Gier & Marion 1968). Inserting a hand through the cervix is usually difficult in normal animals by 24 hours after parturition, and the cervix often only admits two fingers by 96 hours postpartum. By about 3 weeks postpartum, the entire genital tract is usually palpable per rectum. The previously gravid horn can still be identified because it is wider and longer than the previously non-gravid uterine horn. In parallel with the changes in dimensions, there is a remarkable change in the tissues, with catabolism, repair, and regeneration. In the first 30 days postpartum, the weight of the uterus decreases from about 9 kg at parturition to about 1 kg (Gier & Marion 1968).

The progress of uterine involution can be monitored by repeated estimation of the size of the uterus after parturition (Fig. 23.3). The most practical techniques to evaluate the size of the uterus are transrectal palpation and transrectal ultrasonography (Okano & Tomizuka 1987, Sheldon et al. 2000, Sheldon et al. 2003). It should be noted that dimensions estimated by transrectal palpation are often about 1 to 2 cm greater than ultrasound measurements, presumably because operators include the thickness of the rectal wall when using the former to estimate the diameter of the uterus. The size discrepancy between the previously gravid and non-gravid uterine horns is present up to 4 weeks postpartum (Okano & Tomizuka 1987, Tian & Noakes 1991b, Risco et al. 1994). However, the changes in uterine horn diameter are almost imperceptible beyond 4 weeks postpartum and are probably complete by 6 weeks.

The time to completion of uterine involution is often reported in the literature, but this endpoint is rarely used in clinical practice. On the other hand, factors that delay uterine involution are important in clinical practise because delayed involution of the uterus is associated with subsequent impaired fertility (Fonseca et al. 1983). The factors that delay involution include dystocia, hypocalcaemia, RFM, metritis, and endometritis.

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Regeneration of the Endometrium

The epithelium of the endometrium is often damaged during parturition, and inflammation, remodelling, and regeneration of the endometrium is part of the physiology of the postpartum period (Gier & Marion 1968, Wagner & Hansel 1969, Tian & Noakes 1991a). The caruncular tissue undergoes necrosis of the stratum compactum, and by 12 to 14 days postpartum the tissue sloughs as part of the physiological process of the puerperium (see Fig. 7.3). After the caruncular tissue is sloughed, there is reepithelialisation of the caruncles, advancing centripetally from the edges of the caruncle (Wagner & Hansel 1969). Loss of the epithelium is thought to be more likely in animals in which there has been damage during parturition or by microbial infection. As reepithelialisation progresses, the initially flattened epithelial cells develop their columnar appearance, typical of normal endometrium.

Neutrophils and lymphoid aggregates are common in the tissue during the repair of the endometrium, and they are likely to be part of the immune response to tissue damage and infection. Tissue repair is obviously important to counter the negative effects of trauma and as part of the healing process. Genes involved in extracellular matrix homoeostasis, such as the matrix metallopeptidases MMP1, MMP3, MMP9, and MMP13, are differentially regulated in cows with severe negative energy balance compared with more normal animals (Wathes et al. 2009). In most cows reepithelialisation of the caruncles and repair of the endometrial tissue to its normal architecture occur by about 20 to 30 days postpartum (Wagner & Hansel 1969, Archbald et al. 1972).

Return of Ovarian Cyclic Activity

The return of ovarian cyclic activity after parturition requires a coordinated programme of physiological events, involving the hypothalamus, pituitary, ovary, and uterus. Genetic and environmental factors influence these events, and often lead to subfertility; for details of return of ovarian cyclic activity consult Chapters 1 and 7. Briefly, during pregnancy there are high concentrations of oestradiol and progesterone in the peripheral circulation. Within a few days of parturition, the circulating steroid hormone concentrations decrease to basal values. About 7 days postpartum there is an increase in plasma FSH concentration, with subsequent recurrent increases in FSH concentrations every 7 to 10 days (Crowe et al. 1998, Duffy et al. 2000). The first postpartum dominant follicle, characterised by an internal diameter greater than 8 mm, is usually selected about 10 days after parturition. This dominant follicle may ovulate to form the first postpartum corpus luteum, or the dominant follicle may undergo atresia, with subsequent emergence of a second dominant follicle, or the dominant follicle may abnormally persist as a follicular cyst (Savio et al. 1990, Stagg et al. 1995, Beam & Butler 1997). The fate of the first postpartum dominant follicle depends on LH pulse frequency, and failure to ovulate is usually a consequence of inadequate LH pulse frequency and reduced ovarian follicle oestradiol (Beam & Butler 1999, Duffy et al. 2000, Cheong et al. 2016). In dairy cattle, metabolic stress – most often negative energy balance – is the main cause of reduced LH pulse frequency, although a range of other factors can affect ovarian cyclic activity (Cheong et al. 2016).

Of note when examining animals in the postpartum period is that the first dominant follicle is about twice as likely to be in the ovary contralateral to the previously gravid uterine horn. This imbalance in dominant follicle selection is still evident for the second dominant follicle, but the effect of the uterus on the distribution of ovarian dominant follicles is no longer evident beyond the third dominant follicle (Sheldon et al. 2000).

Control of Microbes in the Uterus

Understanding of which microbes are found in the female genital tract has changed markedly over the last 20 years. Previously, it was thought that the uterus was sterile during pregnancy, and that non-specific bacteria from the animal and the environment contaminated the uterus, during and after parturition. However, the uterus has a microbiota and is not sterile. Furthermore, although non-specific microbes from the environment contaminate the uterine lumen during and after parturition, specific microbes that are adapted to the endometrium are key to developing postpartum uterine disease. Outside the puerperium, other specific pathogens, such as *Salmonella* and *Campylobacter* species of bacteria and Infectious Bovine Rhinotracheitis and Bovine Virus Diarrhoea viruses, cause abortion, endometritis, and vaginitis (see Chapter 24).

It is widely accepted that most organs of animals, such as the gut, skin, and vagina, have a community of commensal, symbiotic, and pathogenical microorganisms – often termed the microbiota. There is emerging evidence that the uterus also has a microbiota and is not sterile. Studies using fluorescent probes to image bacteria and 16S ribosomal RNA gene sequencing of endometrium provide evidence that there is a sparse microbiota in the uterus even during pregnancy (Karstrup et al. 2017, Moore et al. 2017). The bacteria identified in these studies include *Trueperella, Fusobacteria* and *Prevotella* species. Although the finding of a microbiota in the uterus is important, the abundance of microbes in the uterine microbiota is substantially less than for the gut, skin, or vaginal microbiota. Furthermore, the abundance of bacteria in the uterine microbiota, outside the puerperium, is also a small fraction of those present in animals with postpartum uterine disease. Whereas many of the bacteria found in the postpartum uterus likely derive from the vagina, skin, gut, and the environment, it is possible that after parturition there is a bloom in the growth of pathogenical bacteria present in the uterus before parturition, which also help establish disease.

Pathogens in the Postpartum Uterus

Multiple species of bacteria are found in the postpartum uterus of cattle. However, as with most infections, disease depends on the balance among the microbes, the environment, and host defences. Most beef breeds and *Bos indicus* cattle are exposed to the same microbes as dairy cattle, yet clinical disease of the uterus is more common in dairy cows.

Postpartum uterine disease is principally associated with the isolation of pathogenical bacteria from the uterus. It is important to note that, unlike many specific clinical diseases, the metritis complex is polymicrobial rather than that found in other tissues in which there is often one-microbe-one-disease. Furthermore, an important concept is that all normal healthy animals have a substantial load of bacteria in the uterus after normal partition. As with many infections, the development of disease depends on the pathogens overcoming host defences. The microbial community in the uterus during the healthy or diseased postpartum period often fluctuates, with cycles of infection, elimination, and reinfection with bacteria. The bacteria most commonly isolated from animals with uterine disease are *Escherichia coli, Trueperella pyogenes, Fusobacterium necrophorum,* and *Prevotella* and *Bacteroides* species (Elliott et al. 1968, Griffin et al. 1974a, Huszenicza et al. 1991, Noakes et al. 1991). However, many additional species of

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TABLE 23.1 Categorisation of bacteria isolated from the postpartum uterus of cattle, according to their potential pathogenicity *The categories are: (1) pathogens known to cause endometrial lesions; (2) potential uterine pathogens; and (3) bacteria not recognised as uterine pathogens that are likely contaminants of the uterine lumen*

Pathogens	Potential Pathogens	Contaminants
Escherichia coli	Acinetobacter spp.	Aerococcus viridans
Trueperella pyogenes	Bacillus licheniformis	Clostridium butyricum
Prevotella spp.	Enterococcus fecalis Haemophilus somnus	Clostridium perfringens
Fusobacterium necrophorum	Mannhiemia haemolytica	Corynebacterium spp.
Fusobacterium nucleatum	Pasteurella multocida	Enterobacter aerogenes
	Peptostreptococcus spp.	Klebsiella pneumoniae
	Staphylococcus aureus (coagulase +)	Micrococcus spp.
	Streptococcus uberis	Providencia rettgeri
	Bacteroidetes species	Providencia stuartii
	Firmicutes species	Proteus spp.
	Fusobacteria species	Proprionobacterium granulosa
		Staphylococcus species
		α-haemolyic Streptococci
		Streptococcus acidominimus

From Sheldon et al. 2002b.
Categories from: Elliott et al. 1968; Griffin et al. 1974a; Ruder et al. 1981; Olson et al. 1984; Huszenicza et al. 1991; Noakes et al. 1991; Dohmen et al. 2000; Sheldon et al. 2002b; Westermann et al. 2010.

bacteria are considered as pathogens, or potential pathogens, of the bovine uterus (Table 23.1).

The importance of pathogens for the postpartum uterus is supported by the ability to develop animal models of endometritis. The role of *E. coli* and *T. pyogenes* is highlighted by infusing *E. coli* and *T. pyogenes* into the uterus of naïve cows to create animal models of endometritis (Ayliffe & Noakes 1982, Amos et al. 2014). Infusion of both pathogens more reliably generates an animal model when using naïve heifers, but infusion of either pathogen also generates disease in cows, with often the second pathogen appearing in the uterine discharge within a week of starting the infusion of the first pathogen. When generating the animal model for endometritis, two additional features will help establish the model. The first is the use of exogenous progesterone, which may suppress immune defences. The second is that disease is more likely if the endometrium is gently debrided immediately before infusion of the bacterial suspension. Presumably, debridement of the surface of the epithelium disrupts the protective epithelium, allowing the pathogens to adhere and invade the underlying stroma.

The importance of specific pathogens causing metritis is also supported by reports of vaccines containing components of *E. coli, F. necrophorum,* and/or *T. pyogenes* that protect animals against metritis (Nolte et al. 2001, Machado et al. 2014). Some of the pathogens appear to be adapted to the endometrium or are specific to the bovine endometrium. For example, novel strains of *E. coli* have been isolated from the uterus of animals with uterine disease (Sheldon et al. 2010). These endometrial pathogenical *E. coli* (EnPEC) are more adherent and invasive for endometrial epithelial and stromal cells than *E. coli* isolated from the uterus of clinically unaffected animals; they stimulate endometrial inflammation, and they establish disease in animal models.

Trueperella pyogenes is the pathogen most associated with the severity of endometrial pathology and clinical disease (Bonnett et al. 1991a, Westermann et al. 2010). The link between *T. pyogenes* and disease probably depends on the cholesterol-dependent cytolysin and pyolysin secreted by *T. pyogenes.* Pyolysin causes cytolysis of a wide range of cells, but endometrial stromal cells are particularly sensitive to pyolysin (Amos et al. 2014, Preta et al. 2015). Pyolysin binds cholesterol-rich domains in the plasma membrane of endometrial cells to form pores in the plasma membrane, which lead to osmotic cell death (Amos et al. 2014, Preta et al. 2015). Endometrial stromal cells are probably particularly sensitive to pyolysin because they have abundant cholesterol. The stromal sensitivity to pyolysin may also explain how *T. pyogenes* switches from being a commensal bacterium in the uterus when the epithelium is intact to causing uterine pathology once the epithelium is breached during and after parturition, thereby allowing bacteria to access the stroma (Amos et al. 2014). Although pyolysin does not stimulate inflammatory responses, *T. pyogenes* does stimulate inflammation, presumably via endometrial cells recognising bacterial lipopeptides (Borges et al. 2012, Turner et al. 2014).

There is evidence for coinfections that foster disease. *T. pyogenes, F. necrophorum,* and *Prevotella* act synergistically to increase the likelihood and the severity of endometritis (Ruder et al. 1981, Olson et al. 1984). More recent studies, using aerobic and anaerobic cultures, confirm the importance of *E. coli, T. pyogenes,* and anaerobic bacteria (Dohmen et al. 2000, Sheldon et al. 2002b, Westermann et al. 2010). Interestingly, there is evidence for an association between *E. coli* and then colonisation by *T. pyogenes* (Williams et al. 2007).

Metagenomics techniques have found associations between uterine disease and bacteria that are not readily cultured by standard techniques (Machado et al. 2012, Santos & Bicalho 2012, Peng et al. 2013, Knudsen et al. 2015, Wagener et al. 2015). Although some of the studies find *E. coli, T. pyogenes* and the expected anaerobic bacteria, others report finding *Bacteroidetes* and *Firmicutes.* There remains a gap in understanding about how the latter 'unculturable' bacteria contribute to the pathogenesis of uterine disease. However, a consistent finding among most microbiology studies

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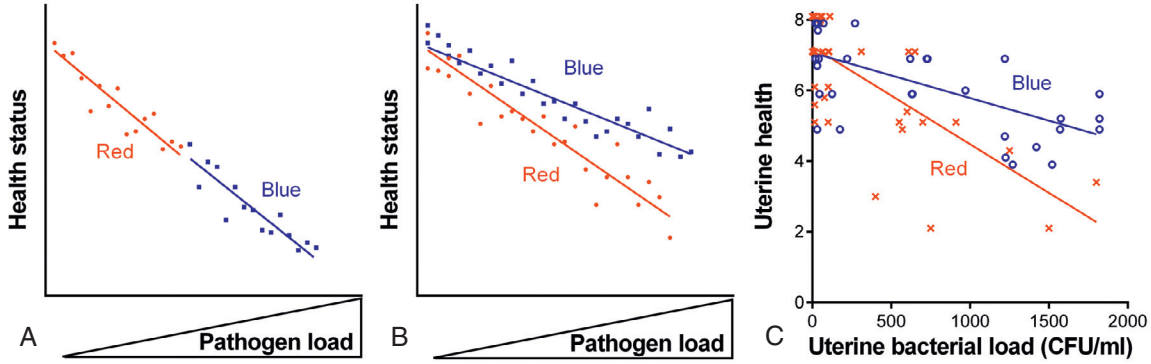

• **Fig. 23.4** (A) Schematic 'reaction norm' of health status against pathogen load for two equally resilient groups of animals. The blue group has *impaired immunity,* with reduced health status and more pathogens, compared with the red group (Data from Raberg et al. 2007). (B) Two groups with similar immunity, but the red group is *less resilient,* with reduced health at the same pathogen load as the blue group (Raberg et al. 2007). (C) The reaction norm of postpartum uterine clinical health score (Sheldon et al. 2002b), against uterine bacterial load for dairy cows, in which a group of metabolically stressed cows producing more than 35 litres/day (x,*red line*; n = 56) were less resilient to infection than a group of animals producing less than 35 L milk/day (o, *blue line*; n = 34).

is that anaerobic bacteria are more abundant in the diseased endometrium than in healthy uteri. Perhaps this is not surprising, as the endometrium is a microaerophilic environment, with tissue damage likely reducing the oxygen tension further. Taken together, the evidence is that *E. coli, T. pyogenes,* and anaerobic bacteria are probably the main pathogens causing the clinical signs of uterine disease but that other pathogens may contribute to postpartum uterine disease.

Viruses and other microbes are rarely associated with nonspecific metritis or endometritis in cattle after parturition. One exception is Bovine Herpesvirus 4, which has been linked to sporadic cases or outbreaks of metritis or endometritis in dairy herds in several countries (Frazier et al. 2001, Monge et al. 2006). The link between Bovine Herpesvirus 4 and uterine disease may be explained by tropism of the virus for endometrial stromal cells and endometrial inflammation associated with viral infection (Donofrio et al. 2007).

Resilience to Infections

The ability to counter pathogenical microbes depends on an organism's *immunity* and *resilience* (Schneider & Ayres 2008, Medzhitov et al. 2012). *Immunity* is the ability to resist microbes and limit the pathogen burden by killing the microbes. *Resilience* is the ability to limit the effect of pathogens on health by tolerating a given microbial burden (Raberg et al. 2007). Failure of both immunity and resilience impair health. Much is known about immunity in the postpartum genital tract. However, resilience is increasingly thought to be important for maintaining animal health (Schneider & Ayres 2008, Medzhitov et al. 2012).

Fifty years ago, dairy cows tolerated the bloom of bacteria found in the uterus after parturition, and they did not develop disease. Now, 20% to 40% of cattle develop uterine disease each year (Sheldon et al. 2009) (see Fig. 23.2). This increased incidence of disease coincides with intensification of farming and breeding dairy cattle for increased milk yields over the last 50 years. Modern cows simply cannot consume enough food to meet the metabolic demand of producing more than 35 L milk/d. Typical metabolic energy requirements have risen from about 100 MJ/d to more than 240 MJ/d (Chagas et al. 2007, Kerestes et al. 2009). Consequently, these animals have reduced blood glucose, glutamine, and metabolic hormones (Doepel et al. 2006, Chagas et al. 2007). It is not clear if this increased postpartum uterine disease is a failure of immunity and/or resilience in cows. However, by comparing the health status of organisms with their pathogen load in populations, it is possible to disentangle the relative contributions of immunity (Fig. 23.4A) and resilience (see Fig. 23.4B) (Raberg et al. 2007). Data from a dairy herd of cows with similar genotype, in which milk production, uterine health, and bacterial load were recorded (Sheldon et al. 2002b), were used to determine whether the metabolic stress of high milk yields in early lactation perturbed uterine immunity or resilience. Postpartum cows under the metabolic stress of producing more than 35 litres milk/day had worse endometrial health with the same uterine bacterial load (see Fig. 23.4C), implying that metabolic stress perturbs uterine resilience. Thus factors that affect the resilience of the uterus are likely to be at least as important as immunity. However, it must be remembered that once pathogens overcome the mechanisms of resilience, then immunity and inflammation have a central role in the progression and the resolution of disease.

Physical barriers are an important feature allowing animals to tolerate pathogens (Fig. 23.5). The obvious anatomical barriers include the vulva, vagina, and cervix, which physically counter microbial infections ascending the reproductive tract to the uterus. However, during and after parturition, the vulva, vagina, and cervix are dilated, and so they are not effective barriers to ascending infections of the genital tract or iatrogenic contamination of the uterus with bacteria.

In humans, *Lactobacilli* species in the vaginal microbiota provide some protection against pathogens, probably by competing for nutrients and generating an acid environment (Ravel et al. 2011). However, the human is unusual in this respect, with cattle having a fraction of the lactobacilli of humans and a less acid vaginal environment (Miller et al. 2016). Whether manipulating the resident microflora of the vagina or the pH of the vagina to counter pathogens and limit uterine disease is in cattle is yet to be verified by clinical trials, but there is evidence

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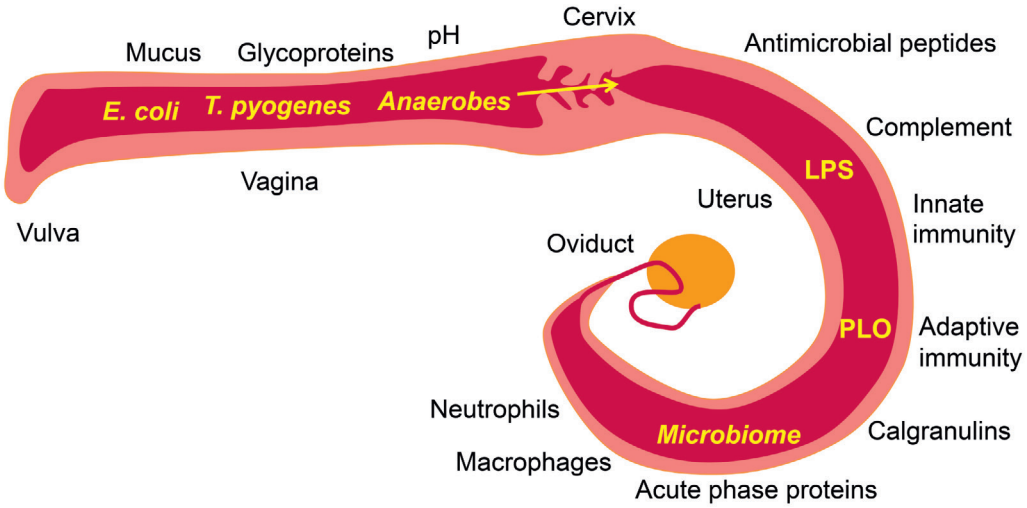

• **Fig. 23.5** Outline of defence systems in the bovine female reproductive tract. (Modified from Sheldon IM. *Reproduction, Fertility, and Development.* 2015;27:72–81.)

that infusion of *Lactobacilli* into the vagina may have a beneficial effect (Deng et al. 2014).

Once pathogens enter the uterus, the ability to tolerate them is dependent on the resilience of the uterine tissues and cells. Most mucosal diseases develop after the epithelium is breached, allowing infection of the stroma. The endometrium is the mucosa of the uterus, and most of the pathology of uterine disease is in the stroma. The first line of defence is the physical barrier of the single columnar epithelium of the endometrium. The tight junctions between epithelial cells separate the apical and basolateral components of the endometrium, and this barrier counters microbial invasion from the uterine lumen. Epithelial cells are also more robust than stromal cells. For example, endometrial epithelial cells are more resistant than stromal cells to pore formation and cell death when exposed to *T. pyogenes* or the cholesterol-dependent cytolysin, pyolysin (Amos et al. 2014). This resilience of the epithelial cells may be because they contain less cholesterol in their plasma membrane than stromal cells. Indeed, reducing cellular cholesterol using methyl-beta-cyclodextrins protects stromal cells against pyolysin (Amos et al. 2014).

The mucus layer on the apical surface of epithelia provides an additional obstacle to microbes. The secretion and character of mucus in the genital tract is under the control of ovarian steroids, and during oestrus, mucus flow increases considerably. In contrast, the cervical mucus plug provides a physical barrier to ascending infections during gestation. The expression of MUC1 is induced when endometrial epithelial cells are treated with endotoxin from *E. coli* (Davies et al. 2008) and in cytobrush samples of the endometrial epithelium of cows with uterine disease (Kasimanickam et al. 2014). Lysozyme expression is also increased in the endometrium of cows with uterine inflammation, and lysozyme digests the peptidoglycans found in the cell walls of bacteria (Hoelker et al. 2012). Antimicrobial peptides and mucosal glycoproteins cover the mucosa of the vagina, cervix, and endometrium, where they also neutralise bacteria and prevent them reaching the plasma membrane of the epithelia. The principal cysteine-rich, cationic, antimicrobial peptides expressed in the bovine endometrium include β-defensins, lingual antimicrobial peptide (LAP), and tracheal antimicrobial peptide (TAP); several gene transcripts for antimicrobial peptides are more abundant in the face of microbial challenge (Davies et al. 2008, Chapwanya et al. 2009).

Although physical barriers and secreted proteins are important for resilience to microbes in the female genital tract, there are some caveats. First, microbes have evolved countermeasures and strategies to avoid many host defences. For example, some bacteria produce enzymes that lyse mucus to penetrate the protective layer, and many bacteria secrete proteases that can disrupt peptides and proteins produced by the host (Baxt et al. 2013). Second, immunity is principally responsible for initiating rapid inflammatory responses to resist microbial infections (Takeuchi & Akira 2010).

Resistance to Infections

Once pathogens overcome the resilience of the uterine tissue, innate immunity and adaptive immunity help resist infections by killing pathogens. Furthermore, much of the endometrial pathological change is a consequence of the inflammation associated with the immune response. Innate immunity is an immediate, non-specific defence system against pathogens, which does not depend on prior exposure to microbes. Adaptive immunity is dependent on antigen-specific receptors, and the response may take several days, depending on whether there has been prior exposure to the antigen. Several studies have examined the inflammatory response to infection in the endometrium. The expression of several cytokines and chemokines are increased in diseased endometrium, including IL1A, IL1B, IL6, CXCL8, CXCL5, TLR4, and PGES (Table 23.2) (Sheldon 2014). Animals with clinical endometritis also have higher concentrations of IL-1α, IL-1β, and IL-6 in uterine fluid than normal animals (Healy et al. 2014, Kim et al. 2014).

Innate Immunity

Innate immunity provides immediate non-specific defence responses against bacteria and tissue damage (Takeuchi & Akira 2010, Moresco et al. 2011). The result is uterine inflammation, influx of immune cells, changes in metabolism, induction of acute phase proteins, activation of the complement system, and other local defence

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TABLE 23.2 Differentially expressed genes in the endometrium or endometrial cytology samples between normal animals and postpartum cows with uterine disease

Ontology	Differentially Expressed Genes	Supporting References
Cytokines	IL1A, IL1B, IL6, TNF, IL12A, IL1R1, IL1R2	Chapwanya et al. 2009; Gabler et al. 2009; Herath et al. 2009b; Fischer et al. 2010; Gabler et al. 2010; Galvao et al. 2011; Ghasemi et al. 2012; Kasimanickam et al. 2014
Chemokines	CXCL5, CXCXL8	Fischer et al. 2010; Gabler et al. 2010; Galvao et al. 2011; Ghasemi et al. 2012; Kasimanickam et al. 2014
Prostaglandins	PTGS1, PTGS2, PTGDS	Gabler et al. 2009; Gabler et al. 2010
Innate Immunity	TLR4, NFKB1	Chapwanya et al. 2009; Herath et al. 2009b; Kasimanickam et al. 2014
Mucins	MUC1	Kasimanickam et al. 2014
Antimicrobial peptides	TAP, DEFB5, DEFB1	Chapwanya et al. 2009
Acute phase proteins	HP, SAA3	Chapwanya et al. 2009
Metabolism	IGF1	Kasimanickam et al. 2014

From Sheldon 2014.

systems. The aim of these responses is to return the genital tract to a state of homoeostasis.

Acute phase proteins are synthesised in the liver, often in response to increased peripheral plasma concentrations of cytokines such as IL-6, and they have important functions in restoring homoeostasis after infection or tissue damage (Baumann & Gauldie 1994). These functions include haemostasis typified by the action of fibrinogen, antimicrobial effects, and the attraction and activation of phagocytes. The severity of bacterial contamination in the postpartum uterus is associated with the concentrations of acute phase proteins in peripheral plasma, including α_1-acid glycoprotein, haptoglobin, and ceruloplasmin, particularly in the presence of uterine infections with *E. coli* and *T. pyogenes* (Smith et al. 1998b, Sheldon et al. 2001). However, the acute phase response is not only initiated by infection but also by trauma (Baumann & Gauldie 1994). Indeed, parturition is a traumatic event, and as uterine involution progresses, there is a decrease in the concentrations of α_1-acid glycoprotein, haptoglobin, and ceruloplasmin (Sheldon et al. 2001). The local expression of genes encoding acute phase proteins has also been noted in the uterus and ovary, which may be of interest because they may provide further localised protection (Chapwanya et al. 2009, Fischer et al. 2010, Lecchi et al. 2012). However, it is possible that the more usual and more abundant hepatic production of acute phase proteins will be more relevant *in vivo.*

Antimicrobial S100 calgranulin proteins and cathelicidin are also more abundant in the endometrium of animals with uterine disease (Wathes et al. 2009, Swangchan-Uthai et al. 2013, Ledgard et al. 2015). The proteins are abundant in neutrophils and are associated with antimicrobial activity, attracting neutrophils to sites of inflammation and stimulating neutrophil activity.

Complement also provides defence against microbial infections. The complement system comprises about 20 proteins, which generate lytic complexes at the surface of pathogens and provide opsonins such as C1b and C3b, which interact with cell surface receptors to promote phagocytosis by neutrophils and macrophages. To avoid complement actions on tissue cells, the latter possess complement regulatory proteins such as CD46, CD55, and CD59 (Gasque 2004). Components of the complement system, such as the genes C1QA, C1QB, C1QC, C3, and C8, are differentially expressed in the endometrium of diseased postpartum cows with more severe negative energy balance (Wathes et al. 2009).

Although the proteins that counter microbes are important, much of the innate immune defence of the uterus depends on the detection of microbes by cellular–pattern recognition receptors. Host cells possess pattern recognition receptors that bind pathogen-associated molecular patterns (PAMPs). These PAMPs are usually highly conserved molecules found in prokaryotes, but not eukaryotes, and include lipopeptides, lipopolysaccharides, and microbial RNA and DNA molecules. The concept is that cellular receptors, such as Toll-like Receptors (TLRs) and NOD-like Receptors (NLRs), bind PAMPs (Takeuchi & Akira 2010, Moresco et al. 2011). For example, TLR2 on cells binds to bacterial lipopeptides, whereas TLR4 binds to endotoxin, which is the lipopolysaccharide cell-wall component of Gram-negative bacteria, such as *E. coli.* In general, the TLRs that sense bacteria are in the plasma membrane (TLR1, TLR2, TLR4, TLR5, and TLR6). Binding of PAMPs to TLRs activates intracellular signalling pathways that results in the production of inflammatory mediators IL-1β, IL-6, IL-8, and prostaglandin E_2. These inflammatory mediators attract and activate immune cells such as neutrophils and macrophages to clear the bacteria (Takeuchi & Akira 2010, Moresco et al. 2011). Other pattern recognition receptors are intracellular and principally detect microbes that invade cells. For example, the release of mature IL-1β is dependent on activation of the 'multiprotein inflammasome complex', often containing NLRP3 (nucleotide-binding domain and leucine rich repeat pyrin 3 domain), leading to caspase-1 activation to cleave pro-IL-1β to mature protein (Schroder & Tschopp 2010).

The receptors used by the innate immune system are principally expressed by hematopoietic cells such as macrophages, dendritic cells, and neutrophils (Takeuchi & Akira 2010, Moresco et al. 2011). However, bovine endometrial epithelial and stromal cells also express most TLR genes (Davies et al. 2008). Furthermore, these epithelial and stromal cells respond to bacteria, endotoxin, and lipopeptides, via TLR1, TLR2, TLR4, and TLR6, by secreting IL-1β, IL-6, IL-8, and prostaglandin E_2 (Herath et al. 2006, Cronin et al. 2012, Turner et al. 2014). Indeed, endotoxin switches endometrial epithelial cell prostaglandin secretion away from the

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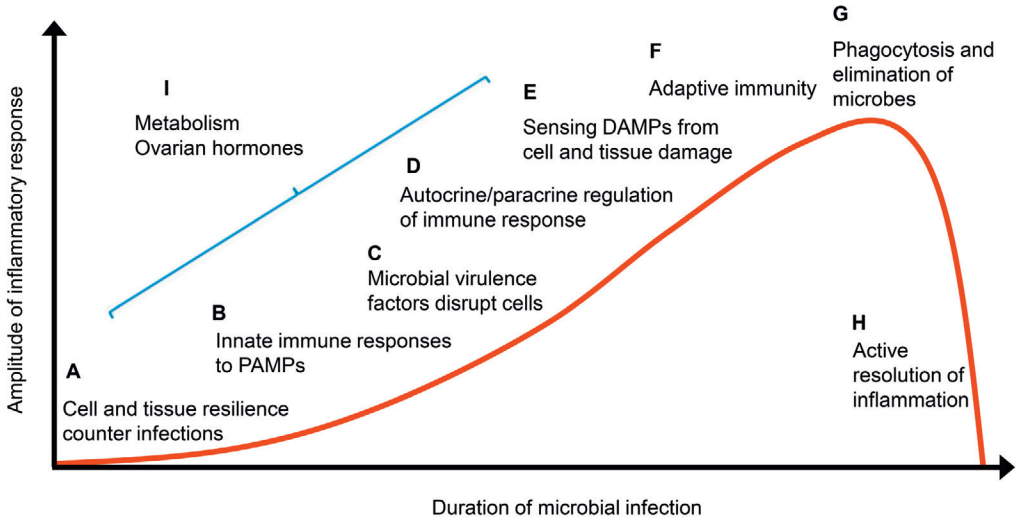

• **Fig. 23.6** A schematic outline of the course of an infection (Sheldon et al. 2017). During an infection, microbes must first overcome the resilience of the endometrial tissue and cells (A). Sensing of PAMPs and vita-PAMPs leads to inflammation, with the secretion of cytokines such as IL-6 and chemokines such as IL-8 (B). The inflammatory response to PAMPs is further increased when host cells additionally sense microbial virulence factors (C), such as pore-forming toxins and bacterial secretion systems. The inflammation is scaled by autocrine and paracrine signalling (D), for example, via IL-6 receptor and STAT3 signalling to endometrial cells. Finally, cell damage and sensing of DAMPs also enhances the inflammatory response (E). Innate immunity also helps direct the adaptive immune response to infection (F). The consequences of inflammation include the immigration of phagocytes to help clear the microbes; phagocytes are attracted to the site of infection along a chemokine gradient and regulated by cytokines such as IL-6 (G). Once the microbes are controlled, there is active resolution of the inflammation and restoration of endometrial tissue homoeostasis (H). All these stages are modulated by hormones, particularly by the ovarian steroid hormones oestradiol and progesterone (I). (Modified from Sheldon, IM, Owens, SE, Turner, ML. *J Reprod Immuno* 2017;119:67–73.)

physiological F series to PGE, and this switch is not overcome by administering oxytocin to mimic the luteolytic signal (Herath et al. 2009a).

The inflammatory cytokines such as IL-6 bind their cognate receptors leading to increased permeability of vasculature and secretion of antimicrobial peptides, eicosanoids, and reactive oxygen species. Cytokines in the peripheral plasma drive systemic inflammatory responses distant to the site of infection; these include pyrexia, generalised vasodilation, and the release of acute phase proteins from hepatocytes. Chemokines such as IL-8 attract and activate neutrophils and monocytes to the site of infection. However, excessive inflammation leads to immunopathology or septic shock, and so innate immunity is carefully calibrated. A series of checks and balances are in place to scale inflammation to meet the level of microbial threat during the progression of an infection (Fig. 23.6) and to limit inflammation when infections are cleared (Blander & Sander 2012). One example in the bovine endometrium is the role of the intracellular signalling molecule STAT3 to regulate the secretion of IL-6 and IL-8 in stromal cells (Cronin et al. 2016). Another example is the apical secretion of IL-6 and IL-8 from bovine endometrial epithelial cells towards the invading pathogens in the uterine lumen and away from the underlying stromal cells (Healy et al. 2015).

Trauma to tissues associated with cell destruction by microbes or by physical damage during parturition may also contribute to inflammation of the endometrium. The ability of pattern recognition receptors to bind damage-associated molecular patterns (DAMPs) may provide an explanation linking tissue trauma to inflammation (Chen & Nunez 2010). Mammalian DAMPs include HMGB1, IL-1α, mitochondrial DNA, and ATP, which are released from dying or damaged cells (Chen & Nunez 2010). However, in the scaling of the inflammatory response, DAMPs are perceived as less of a threat than PAMPs (Blander & Sander 2012). Interestingly, innate immune responses may also be activated by cholesterol-dependent cytolysins or the ion fluxes that they induce (Gurcel et al. 2006, McNeela et al. 2010); this may be important for *T. pyogenes* infections because the bacteria secretes pyolysin, which forms pores in the plasma membrane of endometrial cells leading to leakage of potassium ions (Amos et al. 2014).

Innate immunity is an ancient evolutionary system, and so it is not surprising that it is integrated with other cellular homeostatic and metabolic pathways (Kotas & Medzhitov 2015). Dairy cattle are under metabolic stress after parturition, with reduced concentrations of nutrients and changes in metabolic hormones, including reduced abundance of glucose, glutamine, and insulin-like growth factor 1 (Chagas et al. 2007, Kerestes et al. 2009). Negative energy balance may impair the inflammatory response and clearance of bacteria from the endometrium, leading to chronic endometritis (Esposito et al. 2014). Certainly, the response to pathogen molecules is energetically expensive *in vivo* and *in vitro* (Turner et al. 2016, Kvidera et al. 2017). A striking example is that animals use more than 1 kg of glucose in the first 12 hours after challenge with endotoxin (Kvidera et al. 2017). Furthermore, the depletion of the key cellular nutrients, glucose or glutamine, reduces inflammatory responses by endometrial tissues *in vitro* (Turner et al. 2016, Noleto et al. 2017). If metabolic stress compromises the ability of animals to respond sufficiently to pathogens, the result may be persistence of infections and chronic inflammation.

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Neutrophils are the main innate immune cell-type found in the endometrium during the postpartum period. Uterine disease is associated with negative energy balance and reduced neutrophil function (Hammon et al. 2006, Galvao et al. 2010). Interestingly, the effects of metabolic stress and impaired neutrophil function are even evident before parturition (Hammon et al. 2006). Cows with a severe negative energy balance have persistent uterine inflammation, whereas animals with mild negative energy balance had recovered their energy balance and repaired their endometrium by 2 weeks after parturition (Wathes et al. 2009). Although uncommon, it is interesting to note that cows that are too fat at calving are also at risk of reduced neutrophil function and may develop endometritis (Zerbe et al. 2000). Metabolic status is often evaluated by monitoring body condition score during the postpartum period. An ideal body condition score profile has been proposed, with animals calving at score 3 to 3.5, using the 5-point body condition scoring scale (Chagas et al. 2007). It is important to minimise body condition score loss in the first 7 weeks postpartum to less than half a body condition score point.

Adaptive Immunity

Some microbes that infect the female genital tract, such as *Brucella abortus,* generate robust protective antibody responses, and effective vaccines are available. For postpartum uterine disease, the role of adaptive immunity in the endometrium is more implied than explicit. Areas rich in T cells and B cells are evident in the postpartum endometrium, often as lymphocytic foci within the stroma (Wagner & Hansel 1969, Bonnett et al. 1991b). Adaptive immune responses are also evident in postpartum animals, with increased abundance of antibodies (Dhaliwal et al. 2001). Furthermore, adaptive immunity may play a role in countering uterine infections as preliminary data on vaccines for metritis suggests they provide some protection against disease (Nolte et al. 2001, Machado et al. 2014). However, postpartum endometritis often occurs after successive calvings, and adaptive immunity does not appear to provide long-term protection.

Diseases of the Postpartum Uterus

Risk Factors for Uterine Disease

Uterine disease is most common in *Bos taurus* dairy breeds that have high milk yields, such as Holstein–Friesian cows. Genetic selection for milk yield is often associated with the increased incidence of uterine disease after parturition. However, genetic selection is increasingly focusing on health traits, and future genetic selection of dairy cattle is likely to reduce the risk of uterine disease. The heritability of metritis was reported to be 0.19 to 0.26 for primiparous and second lactation cows, respectively (Lin et al. 1989), although others have found lower heritability of 0.08 for metritis (Zwald et al. 2004).

The environment is an important determinant of susceptibility to several uterine diseases, and many risk factors have been associated with uterine disease. Indeed, environmental factors may be more important than genetic factors. For example, although some polymorphisms in genes associated with immunity have small effects on uterine health in dairy cows, environmental factors such as dystocia, parity, and ketosis are more predictive for uterine disease than the genetic markers evaluated so far (Pinedo et al. 2013). The environmental risk factors are associated with trauma to the female genital tract, disorders of metabolism, or problems with hygiene (Table 23.3).

Trauma

The environmental risk factors most likely to cause uterine disease are associated with tissue damage. Why trauma is important is not

TABLE 23.3 The environment and risk factors for uterine disease

Environment	Risk Factor	Example References
Trauma and tissue damage	Retained fetal membranes	Paisley et al. 1986; Bruun et al. 2002; Kim & Kang 2003; Han & Kim 2005; Dubuc et al. 2010; Potter et al. 2010
	Male calf	Potter et al. 2010
	Stillbirth	Markusfeld 1984; Potter et al. 2010
	Twins	Markusfeld 1984; Dubuc et al. 2010; Potter et al. 2010
	Dystocia	Dubuc et al. 2010; Potter et al. 2010; Pinedo et al. 2013
	Induction of parturition	Markusfeld 1984
	Parity	Markusfeld 1984; Kim & Kang 2003
Metabolism	Milk fever	Bruun et al. 2002; Whiteford & Sheldon 2005)
	Reduced feed intake ante partum	Huzzey et al. 2007
	Ketosis	Markusfeld 1984; Bruun et al. 2002; Dubuc et al. 2010; Pinedo et al. 2013
	Left displaced abomasum	Markusfeld 1984
	Metabolic disorder	Kim & Kang 2003
Hygiene	Calving season	Markusfeld 1984; Bruun et al. 2002
	Angle of vulva	Potter et al. 2010

From Sheldon 2014.

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clear, but trauma may delay uterine involution, keep the cervix open, and allow bacteria to access the underlying stroma below the protective epithelium. Obvious causes of trauma include: dystocia, a large male calf, stillbirths, twins, first parity, and induction of parturition. However, RFM are the most important risk factor for uterine disease (Paisley et al. 1986, Kim & Kang 2003, Potter et al. 2010). The necrotic material associated with RFM provides a favourable environment for bacterial growth in the uterine lumen, and the retained membranes obstruct the physical barrier provided by the cervix, and delay uterine involution.

Hygiene

It is intuitive that the hygiene of the calving environment and the postpartum housing should be important for development of uterine disease. This might be reflected in the association between uterine disease and when or where cattle are housed, or the angle of the vulva that allows faecal contamination of the vagina. However, direct evidence for the importance of hygiene in the postpartum environment is limited, and some studies have found that the apparent level of hygiene is relatively unimportant (Noakes et al. 1991, Potter et al. 2010).

Metabolism

Uterine disease is associated with changes in metabolism after parturition or diseases that disrupt metabolism such as left displaced abomasum. Dairy cows are often under metabolic stress because they cannot consume enough food to meet the substantial extra demand for nutrients that are required for lactation. At the whole animal level the metabolisable energy required every day to produce 40 litres of milk is about 200 MJ, three times the 65 MJ needed for normal resting metabolism. Consequently, postpartum dairy cows lose weight as tissues are broken down to satisfy the dietary energy and protein deficits (Chagas et al. 2007). The animals also develop insulin resistance, resulting in reduced blood concentrations of insulin-like growth factor (IGF-1), glucose, and glutamine; in addition, the mobilisation of fat reserves increases the concentration of ketones such as acetoacetate and β-hydroxybutyrate (Doepel et al. 2006, Chagas et al. 2007, Wathes et al. 2011).

Metabolic stress in postpartum dairy cows may compromise their peripheral blood immune cell function (Cai et al. 1994, Hammon et al. 2006). Various defects in neutrophil function are reported in cows with diseases and disorders, including metritis, after parturition. For example, superoxide production and chemotaxis by neutrophils is lower before partition in cows that develop metritis than normal animals (Cai et al. 1994). Cows with metritis had lower neutrophil myeloperoxidase and cytochrome *c* reduction than cows with normal uterine health around the time of calving (Hammon et al. 2006). Cows that developed uterine disease also had a lower intracellular PMN glycogen level, which was associated with a greater degree of negative energy balance (Galvao et al. 2010).

Glucose and glutamine are the major carbon substrates used by most cells for metabolic energy. Cells demand even more metabolic energy and nutrient supply when immune responses are activated. Thus it is perhaps not surprising that deficits in glucose or glutamine blunt the inflammatory response in *ex vivo* experiments (Turner et al. 2016, Noleto et al. 2017). Limiting the availability of glucose to *ex vivo* organ cultures of endometrium reduces the secretion of IL-1β, IL-6, and IL-8 in response to endotoxin or lipopeptides (Turner et al. 2016). Similarly, depletion of glutamine, in the presence of abundant glucose, reduced the IL-1β, IL-6, and IL-8 response to endotoxin by at least 50% (Noleto et al. 2017). Effective innate immune responses in the endometrium depend on glucose availability, glycolysis, and glutamine abundance. As well as the dependence on the abundance of nutrients, hormones and intracellular regulatory pathways regulate cellular energy homoeostasis. The principal regulator of cellular energy is AMPK, which senses the ratio of AMP to ATP in the cytosol. A homeostatic level of AMPK activation fosters optimal inflammatory responses (Turner et al. 2016).

The effect of ovarian steroid hormones on postpartum uterine function is not clear. Progesterone is considered immunosuppressive, whereas oestradiol may enhance immunity and was used to treat uterine disease (Lewis 2003). However, there are conflicting data, and exogenous oestradiol infused into the bovine uterus during the postpartum period increased the abundance of bacteria in the uterus (Sheldon et al. 2004). Furthermore, the stage of the oestrous cycle, or exogenous progesterone or oestradiol, did not modulate innate immunity in *ex vivo* organ cultures of bovine endometrium (Saut et al. 2014). Similarly, treatment with oestradiol or progesterone, or inhibitors of oestradiol or progesterone nuclear receptors, did not affect gene or protein expression for IL-1β, IL-6, or IL-8 in endometrial cells or macrophages (Saut et al. 2014). Apart from ovarian steroid hormones, the metabolic hormones insulin and IGF-1 are obvious candidates that might link postpartum metabolic stress and immunity, as concentrations of IGF-1 are reduced, and animals are often insulin-resistant (Wathes et al. 2011, De Koster & Opsomer 2013).

When dairy cows mobilise adipose tissue to satisfy the negative energy balance of lactation, there is an increased peripheral plasma concentration of non-esterified fatty acids (Sordillo et al. 2009, Wathes et al. 2012). These fatty acids are metabolised in tissues to acetyl CoA to provide cellular energy, although excess fatty acid oxidation leads to increased production of ketones. During an immune response, tissue cells tend to further exploit fatty acid oxidation to supply nutrients, whereas immune cells exposed to pathogens often increase fatty acid synthesis as part of their inflammatory response (O'Neill et al. 2016). However, apart from cholesterol, there is limited information about the mechanistic dialogue between lipids and immunity in the bovine endometrium.

Most of the cholesterol in cells is contained within the plasma membrane, and the concentrations of this lipid are tightly regulated by cholesterol uptake, by cholesterol efflux transporters, and by cholesterol synthesis (Goldstein & Brown 1990). The first steps in cholesterol synthesis are encompassed by the mevalonate pathway, converting acetyl-CoA to isoprenoids, which are then converted to squalene and, ultimately, to cholesterol. Cholesterol is partially responsible for the fluidity of plasma membranes. Inhibition of squalene synthase can modulate innate immunity in the endometrium and reduce inflammatory responses to endotoxin (Healey et al. 2016). A discussion of the role of cholesterol in endometritis is incomplete without mentioning cellular resilience because many bacteria secrete pore-forming toxins that target cholesterol in plasma membranes. Reducing cellular cholesterol, using methyl-β-cyclodextrin or an endocytosis inhibitor, increases the resilience of stromal cells to PLO (Amos et al. 2014, Preta et al. 2015). Taking the observations together, reducing cellular cholesterol may limit inflammation but increase stromal cell resilience to pore-forming toxins.

Herd Size

It is assumed that uterine disease is not a contagious disease. However, it is notable that larger herds tend to have more uterine disease, and disease is often more common in production systems

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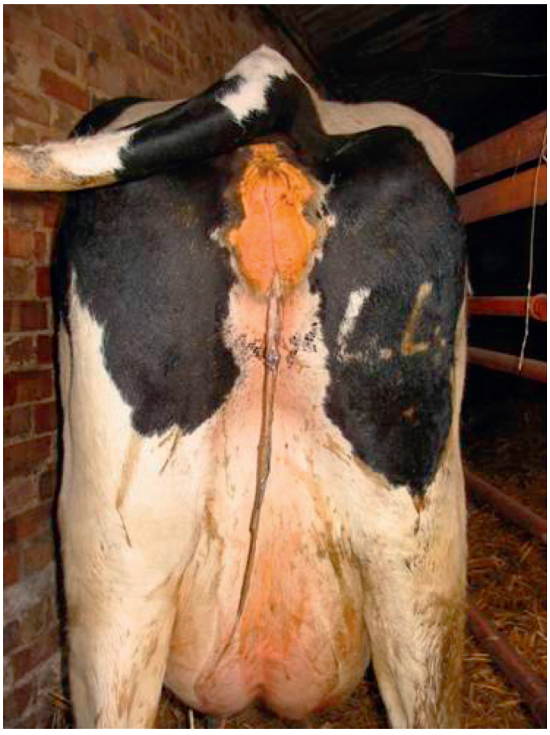
in which animals are confined or managed as large groups. In addition, during experimental infections of naïve animals, disease often develops in the control animals that do not receive an intrauterine infusion of bacteria if they are not kept separate from the animals infused with bacteria.

Retained Fetal Membranes

Retained fetal membranes (RFM) are a common complication of bovine parturition. The predisposition to infections of the uterus means that RFM are an important contributor to bovine infertility. Occurrence of RFM (Fig. 23.7) is associated with the failure of the normal processes of dehiscence and expulsion. Hence the factors that cause RFM are those that interfere with the detachment of the fetal microvilli from the maternal cotyledons and those that interfere with the patterns of uterine contractility, particularly of third stage labour. The main aetiological factors associated with RFM are summarised in Table 23.4.



• **Fig. 23.7** Retained fetal membranes.

Anything that interferes with the process of maturation of the placentomes or causes birth to occur before maturation is complete can result in RFM. Premature birth is very commonly associated with retention. Cattle twins are usually slightly premature and so 30% to 50% of twin births are followed by retention. Premature removal of the calf by Caesarean section and induction of premature calving also cause retention (Gross et al. 1986). Likewise, heat stress can reduce gestation length and increase the incidence of retention of the afterbirth in dairy cattle. Cows that calved during the warm season in Georgia, US, where the mean daily temperature was 26°C, had a reduction of 2.8 days in gestation length and an incidence of retention of 24% compared with 12% for the remainder of the year (DuBois & Williams 1980). The gestation lengths for retaining cows were, on average, 5 days shorter than those of non-retaining cows.

Gross placentitis is also associated with retention of the membranes. Placentitis and RFM occur in cases of abortion due to *Brucella abortus, Salmonella dublin, Campylobacter fetus* and moulds such as *Aspergillus* or *Mucor* species, or genital infections around the time of parturition (Roberts 1986). Retention is also more likely to occur when many cows calve in the same accommodation in quick succession, leading to a buildup of more pathogenical contaminant organisms in the environment. Such outbreaks of retention have also been associated with metritis and calf scour (Roberts 1986). It has been suggested that *T. pyogenes* should only be considered causal of retention when there is evidence of a significant degree of placentitis (Laven & Peters 1996). When placentitis results in retention, it is due to inflammatory swelling of the caruncle and cotyledon, impaired endometrial secretory activity, and impaired myometrial contractility.

Retention also occurs when there is enlargement of the placentomes in the absence of placentitis. Such enlargement may occur in the presence of oedema of the chorionic villi, hyperaemia of the placentomes, advanced involution of the placentomes in postmature fetuses, and prepartum necrosis of the villous tips of the fetal placentome (Paisley et al. 1986, Laven & Peters 1996). These abnormalities may mechanically prevent the separation of fetal and maternal villi.

TABLE 23.4 Factors which predispose to retained fetal membranes

Abortion, especially when the cause is associated with placentitis	
Abnormal gestation length; either prolonged or shortened	
Dystocia, primary uterine inertia, delivery by Caesarean section	
Fatty liver, possibly because it predisposes to uterine atony	
Deficiencies of selenium/vitamin E or, possibly, vitamin A	
Failure of placental maturation	Twin births and induced-calvings are commonly associated with retention of fetal membranes, as the placenta had not completed the maturational changes that are required for normal separation. Heat stress, which often results in shortened gestations, can also result in increased incidences of retained fetal membranes.
Abnormalities of oestrogen; progesterone ratio in late gestation	
Secondary inertia caused by hypocalcaemia is weakly associated with RFM, which is probably because of the association of both with dystocia	

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Uterine inertia, particularly when it results in inadequate uterine contraction during the period of third stage labour, is variably associated with retention. Early studies associated impaired uterine contractility with retention, especially when secondary uterine inertia occurs as a result of dystocia (Jordan 1952, Venable & McDonald 1958). Not all studies agree with this view, however, although inertia may be associated with a retardation of delivery, the membranes have undergone normal maturation (Zerobin & Spörri 1972, Martin et al. 1981, Paisley et al. 1986). It is likely that few cases of retention are caused by uterine inertia and that, even when inertia occurs, detachment of the placenta is easily accomplished. On the other hand, dystocia and fetotomy are consistently associated with retention, to which inertia is likely to be a significant contributor (Wehrend et al. 2002, Eiler & Fecteau 2007). Uterine inertia caused by gross overstretching of the myometrium, such as in animals with hydrallantois, has been more definitely associated with retention (Arthur & Bee 1986), although the abnormalities of placenta and fetus in such animals suggest that other factors are likely to be involved. Uterine inertia due to hypocalcaemia has also been associated with retention (Arthur & Bee 1986, Gröhn et al. 1990, Wilde 2006). However, intervention studies, in which cows have been given calcium as a prophylactic or treatment for RFM, have generally not supported the view that hypocalcaemia has a critical role in the pathogenesis of the disease (Hernandez et al. 1999, Morton 2000, Melendez et al. 2003).

There is evidence of a higher incidence of RFM when cow diets are deficient in selenium and/or vitamin E (Trinder et al. 1973, Julien et al. 1976, Aréchiga et al. 1994, Allison & Laven 2000, Bourne et al. 2007). Correction of dietary deficiencies or supplementary feeding of selenium or vitamin E is commonly associated with a reduction of the incidence of retention (Wilde 2006). Nonetheless, some other studies found no reduction in RFM after prepartum supplementation with selenium (Gwazdauskas et al. 1979, Hidiroglou et al. 1987). Hence selenium deficiency may be a cause of a high incidence of RFM in certain deficient areas, but sporadic cases of retention are probably not associated with selenium deficiency.

Finally, a number of other factors have been associated with RFM. There is some evidence of a hereditary predisposition to RFM. Cows of the beef breeds are much less often affected than those of dairy breeds; in the latter the incidence is higher in Ayrshires than in Friesians. Old cows are more commonly affected than young ones. Springtime calving exerts a predisposing influence that might be connected with a vitamin A deficiency, which has been shown to produce retention under experimental conditions. Animals that have previously had RFM are more likely to do so again (Eiler & Fecteau 2007). The incidence of retention is higher in genetically high yielding dairy cows. In addition, cows on high nutritive planes at parturition are more prone to retention, as are cows with disorders of carbohydrate metabolism, such as fat cow syndrome, ketosis, or displaced abomasum, around the time of calving (Whitmore et al. 1974, Melendez et al. 2003).

Diagnosis and Incidence

Surveys of the incidence of RFM usually report an incidence of 4% to 8%, except in animals with dystocia, in which the incidence is higher. The effect of RFM depends largely upon the degree of uterine infection that takes place. Uncomplicated cases may be of little more consequence than the inconvenience of foul smelling membranes interfering with milking, although morbidity, as denoted by some temporary impairment of appetite and reduction of milk yield, has been estimated to occur in 55% to 65% of cases. In an important early investigation of the morbidity of RFM, it was observed that the pathogenicity of retention on appetite in 44 cattle (Palmer 1932) during the 14 days after calving resulted in appetite being good in 31.8%, fair in 54.5%, and poor in 13.6% of animals.

Mortality caused by RFM is usually associated with metritis and applies to 1% to 4% of cases (Roberts 1986). Cows with RFM that have calved spontaneously after a normal length of gestation generally depart little from normal health. On the other hand, when retention follows extensive obstetrical interference for dystocia, a severe metritis and toxaemia can supervene within 2 to 3 days, which can be fatal if untreated. Whether these cases can be directly attributed to the retention is, however, unclear because similar cases might have been equally ill if the fetal membranes had been expelled at the time of delivery. Clinical signs and treatment of metritis are discussed later in this chapter.

Duration of Retention

Cows that fail to expel the membranes within 24 hours or so are likely to retain them for 7 to 10 days. Myometrial contractions largely cease from 36 hours after the birth of the calf, so if the membranes have not been expelled by this time, freeing of the fetal villi from the maternal crypts eventually occurs as a result of bacterial putrefaction. This process starts within 24 hours of birth but takes several days to complete. Natural sloughing of the maternal caruncles also contributes to the subsequent dehiscence of the membranes, such that eventual expulsion of the membranes depends on uterine involution. The duration of retention seems to depend on several factors, such as the extent of the areas of attachment of the fetal membranes, the rate of uterine involution, the amount of uterine exudate, and the proportion of the afterbirth that had already passed through the cervix when retention began.

Effects on Fertility

RFM is not of itself associated with impaired subsequent fertility. This was first demonstrated by comparing the fertility of 44 cows with RFM, with 44 cows in the herd that had cleansed normally, finding that there was no significant difference in the subsequent breeding records of the two groups (Palmer 1932). A consensus has developed, which supports the idea that uncomplicated retention does not significantly affect the fertility of cows that are mated beyond 60 days from the last calving. The significance of retention is, therefore, dependent on the degree of metritis that occurs. This aspect of the condition was clarified by means of a retrospective analysis of 652 parturitions of 293 dairy cows in Canada, which revealed that RFM alone did not impair subsequent reproductive performance (Sandals et al. 1979). The animals that developed the metritis complex, with or without RFM, did, however, suffer significant increases in days open, services per conception, calving to first oestrus interval, and days from calving to first service. Various studies have confirmed these findings (Joosten et al. 1988, Borsberry & Dobson 1989, Esslemont & Peeler 1993, Kossaibati & Esslemont 1997, Morton 2000). The economic costs of a case of RFM have been estimated as GBP£300 to GBP£475 (Joosten et al. 1988, Kossaibati & Esslemont 1997).

Treatment and Prognosis

The treatment of animals with RFM has long been a contentious subject. A number of approaches have been taken to animals with this condition, including:

- No treatment;
- Manual removal;

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- Administration of ecbolic agents; and
- Treatment for metritis but no specific treatment of retention itself.

Manual Removal

The techniques used for manual removal of RFM range from externally applied gentle traction through to forced extraction, with manual separation of each cotyledon and caruncle. Manual removal is a superficially attractive method in that it immediately removes the stinking mass of putrefying afterbirth, thereby improving milking hygiene. However, manual removal may have little benefit or be detrimental for the animal. In a study that examined 501 cases of RFM, manual removal did not reduce the risk of metritis or endometritis and did not improve fertility compared with other treatments (Drillich et al. 2006b). The authors recommended that systemic treatment, based on elevated rectal temperature, was the optimal choice for treating RFM.

Forced extraction, once commonly practised, is now generally regarded as not advisable. An exception is when release of the membranes is simply impeded by constriction of the cervix or when there are only one or two remaining attachments to the maternal caruncles. Forced extractions of extensively attached fetal membranes can result in damage to the endometrium, and more distant parts of the membranes are often left behind (Grunert & Grunert 1990). If fetal cotyledons remain attached after forced traction, they may become detached later and could remain within the uterine lumen as foreign bodies (Roberts 1986). Similarly, the prevalence and severity of uterine infection are often worse after manual removal than after conservative treatment (Roberts 1986, Bolinder et al. 1988). Some suggest that the presence of pyrexia was an absolute contraindication to the forced removal of fetal membranes (Roberts 1986). Moreover, forced extraction is commonly associated with impaired subsequent reproductive performance (Bolinder et al. 1988).

The current recommendations for the manual removal of fetal membranes are that cows should not be examined until 96 hours after calving and that, if attempted, removal should be gentle, ideally limited to the withdrawal of the membranes from the genital tract after they have become spontaneously detached from the caruncles. Although in many animals, spontaneous detachment may have occurred within 96 hours, it is suggested that it is acceptable to leave membranes for 10 days before removal if this length of time was needed for their detachment (Roberts 1986). Even with minimal intervention, there appears to be no benefit from removal of the membranes (Laven & Peters 1996, Drillich et al. 2006b). In this context, farmers should also be discouraged from attempting to undertake forced removal of fetal membranes from their own cows because they are very likely to use too much force and to attempt removal too soon after calving.

Ecbolic Agents

Many attempts have been made to reduce the incidence of RFM or to hasten the release of the membranes once retention has occurred with ecbolic agents such as oxytocin or $PGF_{2\alpha}$. Oxytocin treatment has little or no beneficial effect (Miller & Lodge 1984, Garcia et al. 1992, Stevens & Dinsmore 1997) even when the uterus has been presensitised by the administration of oestrogenic substances (Arthur & Bee 1986). Similarly, a longer acting form of oxytocin, carbetocin, had no benefit for subsequent fertility when given to dairy cows immediately after parturition (Barrett et al. 2009). Prostaglandin $F_{2\alpha}$ and its analogues have also been used as ecbolic agents in postpartum cattle. Prostaglandins may assist in detachment of the membranes through direct actions upon the placentomes rather than just by an ecbolic action (Gross et al. 1986).

Treatment for Metritis Only

Uncomplicated cases of RFM require no immediate treatment. However, those cows with signs of metritis, such as pyrexia, inappetence, or reduced milk yield, need to be treated with antibiotic. A number of subsequent studies have confirmed that this is an appropriate treatment regimen (Drillich et al. 2006a, Drillich et al. 2006b). The use of parenteral antibiotic for the treatment of cows with metritis is discussed later.

Traditional practice has been that, after forced extraction of RFM or after unsuccessful attempts at extraction, antibiotics were placed into the uterus in an attempt to prevent endometritis. Intrauterine antibiotics reduce odour (Roberts 1986). However, antibiotics also reduce the rate of putrefaction of the membranes and the level of intrauterine phagocytosis, thereby prolonging retention (Paisley et al. 1986, Roberts 1986). Administration of intrauterine antibiotics to cows when they have retained membranes does not reduce the incidence of endometritis or improve fertility (Goshen & Shpigel 2006, Eiler & Fecteau 2007). Furthermore, some of the antibiotics that are present in intrauterine pessaries are inactivated in the presence of the debris that is contained within the uterus (Paisley et al. 1986), a problem that is often exacerbated by veterinarians failing to use the recommended dose. Hence there appears to be little to recommend the use of intrauterine antibiotics when retained membranes are present (Paisley et al. 1986, Goshen & Shpigel 2006, Beagley et al. 2010), although there are potential benefits for cows with metritis (Goshen & Shpigel 2006).

All cows that had RFM require examination and treatment for endometritis before breeding, ideally towards the end of the voluntary wait period (see Chapters 22 and 25). Diagnosis and treatment of endometritis is discussed later in the present chapter.

Collagenase

Infusion of collagenase solution into the stumps of the umbilical arteries of the retained membranes has proved an effective means of treatment (Eiler & Fecteau 2007, Beagley et al. 2010). Although not licensed for use in cattle, collagenase is used successfully to treat RFM in the mare (see Chapter 31).

Metritis

Metritis usually follows an abnormal first or second stage of labour, especially when there has been a severe dystocia that has required prolonged traction or resulted in damage to the vulva and/or birth canal. The condition is also associated with uterine inertia, premature calving including abortion or induced calving, twin births, and RFM. Bacteria colonise and proliferate in the uterus and may cause pyaemia. Toxins produced by these bacteria are also absorbed from the uterus, resulting in toxaemia.

Diagnosis and Incidence

Metritis is more common in dairy and beef breeds of cattle and usually occurs within 10 days of parturition. Metritis is characterised by an enlarged uterus and a watery, red-brown fluid to viscous, off-white, purulent uterine discharge (Fig. 23.8), which often has a fetid odour. The severity of disease is categorised by the signs of health:

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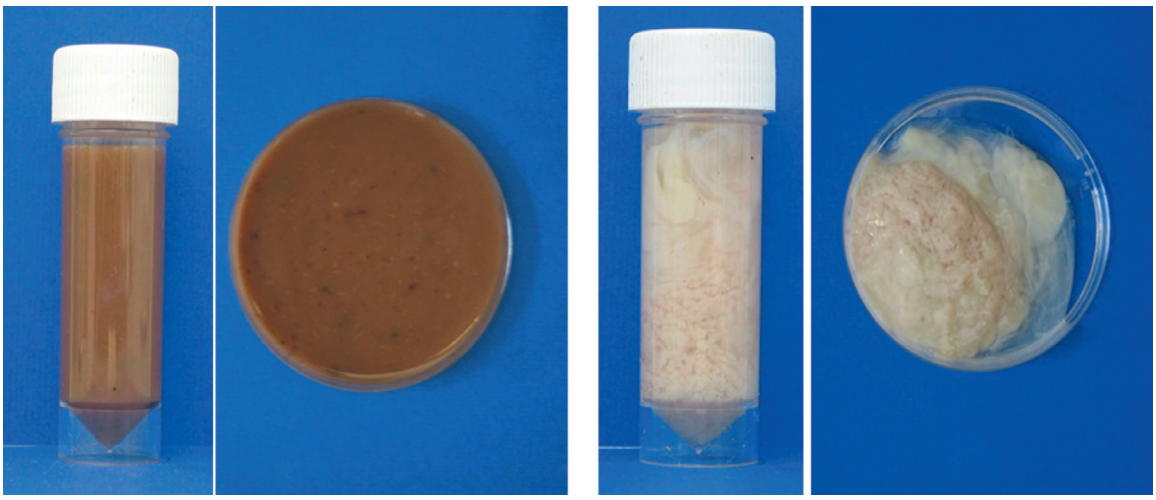

• **Fig. 23.8** Animals with metritis have pus in the uterus that ranges from red-brown watery exudate that contains fragments of degenerating fetal membranes to off-white mucopurulent material.

- Grade 1 metritis: animals with an abnormally enlarged uterus and a purulent uterine discharge, but without any systemic signs of ill health;
- Grade 2 metritis: animals with an abnormally enlarged uterus and a purulent uterine discharge, with additional signs of systemic illness such as decreased milk yield, dullness, and fever; and
- Grade 3 metritis: sometimes called puerperal metritis or toxic metritis; animals with an abnormally enlarged uterus and a purulent uterine discharge, with signs of toxaemia such as inappetence, cold extremities, depression, and/or collapse.

Affected animals show both local and general symptoms, and the severity informs the metritis grade. The temperature of affected cows may be elevated to 40°C to 41°C initially but is often subnormal by the time veterinary attention is sought. Toxaemia or endotoxic shock result in clinical signs that may include: a fast pulse in the region of 100 beats per minute; rapid respiration; a sluggish capillary refill time; cold extremities; a moderate to severe level of dehydration; and a characteristic toxaemic diarrhoea. The vulva and vagina are typically swollen and deeply congested. The cotyledons are swollen and the fetal membranes often remain firmly attached. The uterus contains a large volume of pus; whereas off-white, mucopurulent material is often an encouraging sign, red-brown, watery, fetid, reddish, serous exudate that contains fragments of degenerating fetal membranes and other detritus warrants a more guarded prognosis (see Fig. 23.8). This fluid is discharged from the vagina accompanied by frequent expulsive straining efforts. Vaginal exploration of an affected case should be cautious, as it causes acute discomfort and is followed by severe and persistent expulsive efforts. Many animals with grade 3 metritis are recumbent by the time veterinary attention is sought, by which time they are usually anorexic. In these animals it is common for the infection to extend through the uterine wall into the peritoneum, causing a localised or generalised peritonitis. Many animals with grade 3 metritis also develop mastitis, particularly if they are recumbent, and many also have concurrent hypocalcaemia. The main differential diagnoses are: metabolic disorders; ruptured uterus; retained fetus; diffuse peritonitis; acute toxic mastitis; salmonellosis; and postcalving injuries (e.g., obturator paralysis).

The incidence of metritis varies between breed, country, and herd, but in a study of the records from 97,318 dairy cows in the US, the lactation incidence of metritis, including RFM, was 21% (Zwald et al. 2004). In a meta-analysis of records from more than 10,000 animals, there was evidence that postpartum metritis caused subfertility by increasing the time to first insemination by 7.2 days, reducing conception rate to first insemination by 20%, and increasing the calving to conception interval by 18.6 days (Fourichon et al. 2000). In some studies, metritis is also associated with reduced milk yields (Giuliodori et al. 2013).

Treatment and Prognosis

Grade 1 cases are often undiagnosed and untreated. Routine examination of postpartum cows is used in some herds to identify these animals. However, whether treatment is warranted is unclear. Parenteral antibiotic may help resolve the clinical signs but probably has little effect on health or subsequent fertility.

Grade 2 cases may require the parenteral administration of broad spectrum antibiotics, in combination with other supportive and symptomatic treatments, as appropriate to the severity of the condition. These grade 2 metritis cases warrant treatment, not least because metritis is painful (Stojkov et al. 2015). However, a review of 17 studies using ceftiofur to treat metritis found that although seven reported clinical improvement, there was no significant improvement in reproductive performance (Haimerl & Heuwieser 2014). Ampicillin trihydrate is also used to treat metritis. Ampicillin was at least as effective as ceftiofur in a study of 528 cows with metritis (Lima et al. 2014). If used, parenteral antibiotics should be continued for 3 to 5 days. Oestrogens are prohibited in many countries, and even when available, oestrogens are contraindicated as they increase the rate of absorption of endotoxin from the uterus. Oxytocin may be of some benefit, but only within 72 hours of calving. Prostaglandin $F_{2\alpha}$ is of little or no benefit despite a short-term ecbolic effect.

If the uterus contains an abundance of pus, uterine lavage and drainage can be attempted (Fig. 23.9). Physiological saline is flushed into the uterus using a wide-bore tube inserted through the cervix, with the end of the tube guarded by the operator's hand. Relatively small amounts of saline should be infused at a time as the uterus is too friable to cope with any buildup of pressure. The fluid is then syphoned from the uterus by lowering the tube and the process repeated several times. Although this procedure is commonly undertaken, it should be noted that it is associated with significant risks of uterine wall damage and toxin absorption as the uterine wall may be extremely friable. Intrauterine antibiotics are

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• **Fig. 23.9** Uterine lavage involves repeated cycles of infusing saline into the uterus (A), followed by syphoning the purulent suspension from the uterus (B).

inadequate as the sole route of antibiotic administration, although there may be some advantage to the infusion of soluble antibiotics, such as 5 g oxytetracycline in 100 to 500 mL normal saline, once uterine flushing is completed.

Even if animals are not treated, it is important to record the presence of disease because even grade 1 and 2 metritis cows should be examined for clinical endometritis 21 days or more after parturition.

Grade 3 cases of metritis are serious, and the prognosis should be guarded. Prognosis is poor if the animal is collapsed or if peritonitis is present. In this situation, before initiating treatment, a decision should be made whether the animal has a reasonable chance of recovery or whether it would be more cost effective, in some commercial production systems or from a welfare perspective, to slaughter the animal.

Treatment of grade 3 metritis requires both good nursing care and vigorous medication. As for most cases of sepsis, supporting the animal's physiology is probably more important than trying to kill the pathogens. The cow should be kept warm and made as comfortable as possible by, for example, transferring it to a well-bedded loose box. The animal should be fed and provided with water. The first step in treating these cases is to stabilise the circulatory system by giving fluids and non-steroidal antiinflammatory drugs (Smith 2005). Fluids can be given as 2.0 to 2.5 litres of 7% v/w saline intravenously followed by 25 litres of water administered by stomach tube, or 24 to 40 litres of isotonic electrolytes by stomach tube (Frazer 2005). Flunixin meglumine is probably the non-steroidal antiinflammatory drug of choice because of its antiendotoxic effects. Toxaemia is energetically expensive: animals use an extra kilogramme of glucose in the first 12 hours after an intravenous challenge with endotoxin (Kvidera et al. 2017). So, encouraging animals to eat and, perhaps, intravenous infusion of 500 mL of 50% glucose are beneficial for survival. Calcium should be given to treat or prevent secondary hypocalcaemia. Fetal membranes should not be removed, and vaginal examination should be very cautious. If the cow is continually straining, caudal epidural anaesthesia can be given, but this only gives transient relief for 1 to 2 hours. However, sometimes an epidural will break the cycle of pain and straining, which is often self-perpetuating and debilitating for the animal.

Parenteral antibiotic therapy should be initiated immediately, as for any cause of sepsis, and continued for 3 to 5 days. The choice of antibiotic remains contentious**,** and there are many arguments put forward in favour of one antibiotic over another, with little help from large scale clinical trials. Some favour bactericidal antibiotics to control systemic infection and pyaemia. Broad spectrum penicillins and cephalosporins are the most efficacious choices. Ceftiofur achieves concentrations in uterine tissue and fluid that exceed the minimum inhibitory concentration (MIC) for most of the common metritis pathogens (Drillich et al. 2006a) and appears to be effective in treating acute postpartum metritis (Chenault et al. 2004). In some studies there was also an improvement in subsequent fertility (Giuliodori et al. 2013). Some clinicians prefer bacteriostatic antibiotics as they limit the risk of further endotoxic damage during the killing of bacteria. Oxytetracycline has the advantage that it can be given at high dose intravenously, but the MIC of tetracycline for microbes causing intrauterine infections is often high**,** and sufficient concentrations of oxytetracycline are probably not attained in uterine tissue or the lumen (Smith et al. 1998a).

Recovery of animals with grade 3 metritis is marked by a return of appetite, cessation of diarrhoea, and a change in the contents of the uterus to a less fetid, thicker, more obviously purulent material. The major sequelae of this condition include localised or generalised peritonitis, ascending infection of the urinary tract

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(cystitis or pyelonephritis), pyosalpinx, and ovariobursal adhesions. Other complications of metritis include pneumonia, polyarthritis, and endocarditis. In pyaemic cases, abscesses may develop subsequently in the lungs, liver, kidney, or brain. The fertility of cows that recover from the condition is likely to be impaired, so animals should be monitored for subsequent endometritis and ovarian dysfunction.

Endometritis

Endometritis is a common condition of the cow. Unlike metritis, endometritis does not affect the general health of the cow, although it does impair fertility. Most of the specific pathogens causing infertility, such as *Campylobacter fetus* subsp. *venerealis* and *T. fetus,* do so because of the endometritis that they produce (see Chapter 24). However, the most common initiating cause of endometritis is a polymicrobial growth of bacteria in the uterus during the period around the time of calving, with subsequent overgrowth of pathogens such as *T. pyogenes,* usually in association with *F. necrophorum* and/or *Prevotella* species.

Clinical endometritis increases the interval to first insemination by 11 days and delays conception by 32 days, compared with animals that did not have endometritis (Borsberry & Dobson 1989). Cows with clinical endometritis between 20 and 33 days postpartum are 1.7 times more likely to be culled for reproductive failure than cows without endometritis (LeBlanc et al. 2002a).

Diagnosis and Incidence

Worldwide figures for the incidence of endometritis are varied, ranging from 35% to 45% in some studies, to other studies that report lower incidences of 6% to 10% (Fonseca et al. 1983, Andriamanga et al. 1984, Martinez & Thibier 1984, Borsberry & Dobson 1989). Typically**,** the incidence is 15% to 20% of animals. For example, a survey of 19,870 Holstein cows in Germany found a lactation incidence of 19.2% for endometritis (Gernand et al. 2012).

Clinical endometritis is characterised by the presence of a white or whitish yellow mucopurulent vaginal discharge in the postpartum cow; the condition is also known as leukorrhoea or whites. The volume of the discharge is variable but frequently increases at the time of oestrus, when the cervix dilates and there is copious vaginal mucus. Affected cows do not show signs of systemic illness. Subclinical endometritis is characterised by inflammation of the endometrium, typically associated with neutrophils in uterine luminal fluid, but without visible purulent material.

The presence of endometritis can be determined by transrectal palpation of the uterus, examination of the vagina and cervix for the presence of purulent discharge, and examination of cervical swabs for the presence of neutrophils. The risk of endometritis can also be inferred from a history of relevant aetiological factors in the peripartum period of individual cows.

Rectal palpation of animals with clinical endometritis frequently reveals delayed uterine involution, and a uterus that has a 'doughy' feel. There are correlations between the size and texture of uterus and cervix, the nature of the purulent exudates, and the degree of endometritis determined by biopsy, and the nature of the bacterial isolation (Studer & Morrow 1978). Nonetheless, rectal palpation is now regarded as being neither a sensitive nor a specific method for accurate diagnosis of endometritis as too many other factors interfere with the rate of change of size of the uterine horns over the postpartum period (de Boer et al. 2014). Hence reliance should not be placed upon this method as the primary means of diagnosis.

A simple grading system based on the character of the vaginal mucus is readily used to evaluate cows with clinical endometritis (Fig. 23.10A) (Sheldon & Noakes 1998, Sheldon et al. 2009). The endometritis grade correlates with the presence of pathogenical organisms associated with uterine disease (see Fig. 23.10B) and is prognostic for the likely outcome of treatment (see Fig. 23.10C).

The presence of purulent discharges can be diagnosed in a number of ways. Examination of the vagina with a clean gloved hand is simple, cheap, and effective, but some operators may cause discomfort to the cow. Although somewhat invasive, manual examination of the vagina carries little risk of further microbial contamination of the uterus in postpartum dairy cattle (Sheldon et al. 2002a). Manual examination of the vagina also facilitates collection of fluid from the vagina to evaluate the presence and odour of pus, which can be used to score the severity of disease and predict the likely success of treatment (Sheldon et al. 2006). Vaginal examination also allows the operator to detect damage to the wall of the vagina and cervix, indicative of obstetrical injuries, vaginitis, and cervicitis. However, as with any clinical examination, the evaluation of uterine disease is subjective, and there is interoperator and intraoperator variation (de Boer et al. 2014, Sannmann & Heuwieser 2015). Alternatively, a vaginoscope can be used to examine for the presence of discharge around the cervical os or in the cranial vagina. The most recently developed method is a rubber diaphragm on the end of a stainless steel rod (Metricheck), which is inserted into the vagina to collect a sample of discharges from the external cervical os/cranial vagina. This method is as effective as vaginoscopy but has the advantage of being quick and causing minimal discomfort to the cow. The Metricheck instrument and examples of purulent discharges collected during its use are shown in Fig. 23.11.

The absence of pus in the postpartum genital tract does not mean that the tract is normal and healthy. The importance of subclinical endometritis has emerged over the last 15 years, with the realisation that cytological evidence of inflammation of the endometrium is associated with reduced fertility (Kasimanickam et al. 2004, Gilbert et al. 2005). The cause of subclinical endometritis is not yet clear and may include resolving bacterial infections, immunopathology without pathogenical bacteria, or even aberrations of postpartum tissue regeneration and repair. Subclinical endometritis is characterised by inflammation of the endometrium that results in a significant reduction in reproductive performance in the absence of signs of clinical endometritis.

The Australian 'InCalf' survey of reproductive performance of dairy cattle (Morton 2000) identified a number of epidemiological factors that were associated with subfertility due to endometritis (Table 23.5). Identification of the cows in which these events occur allows them to be managed and/or treated appropriately before they are mated. The term 'at risk cows' has been proposed for these animals.

In the absence of a practical gold standard for the diagnosis of clinical endometritis, there is limited agreement among the diagnostic methods (de Boer et al. 2014). Beyond the clinical diagnosis, studies that use a reproductive outcome to determine the diagnostic criteria are most useful on farms.

Effects on Fertility

Endometritis reduces fertility by reducing the chances of conception. Consequently, there is an increase in the calving–conception interval, the number of services per pregnancy, and the proportion of cows that fail to conceive. Extension of the calving–conception

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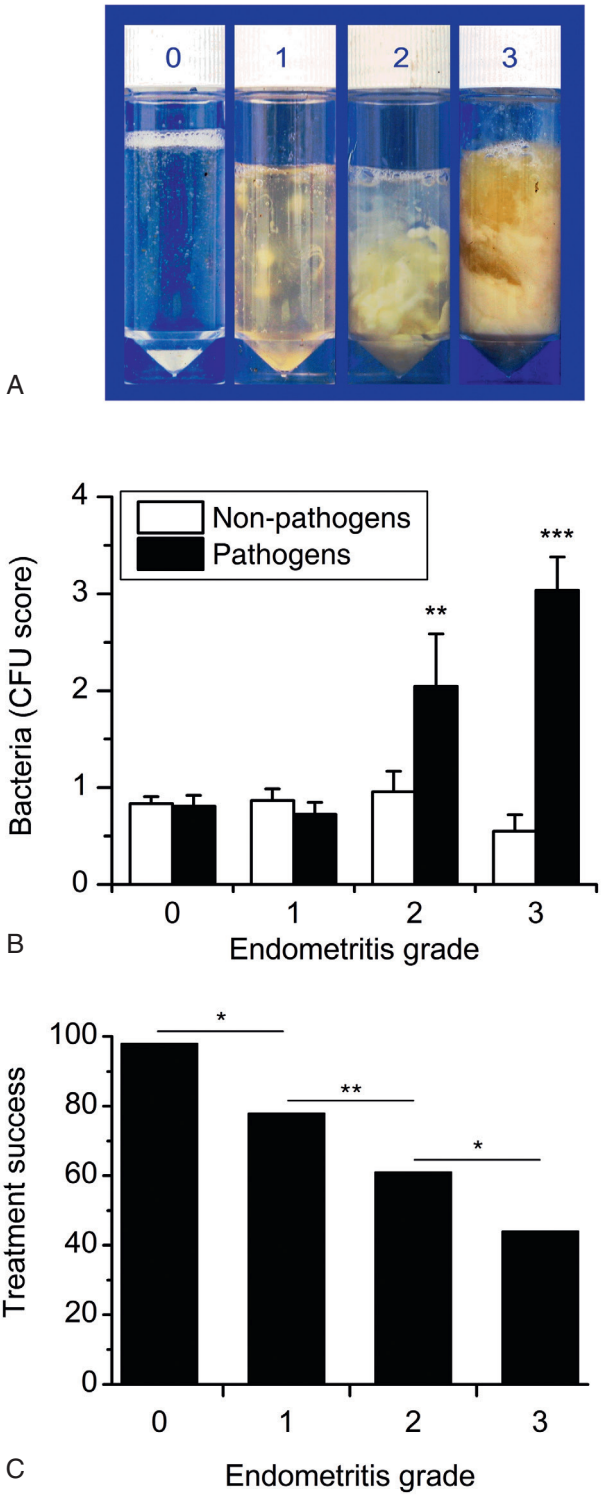

• **Fig. 23.10** Grading scheme for clinical endometritis (Sheldon et al. 2009). (A) Vaginal mucus character is graded as 0 (clear or translucent mucus), 1 (mucus containing flecks of white or off-white pus), 2 (exudate containing < 50% white or off-white mucopurulent material), or 3 (exudate containing > 50% purulent material, usually white or yellow but occasionally sanguineous) (Sheldon et al. 2006). (B) Endometritis grades reflect the number of pathogenical (black bars) but not opportunist non-pathogenical (white bars) bacteria isolated from the uterus of cattle (Williams & Sheldon 2003); data are presented as semiquantitative scores of the number of colony-forming units (CFU) from uterine swabs, in which CFU is scored as 0 (no growth), 1 (< 10 CFUs), 2 (10–100 CFUs), 3 (101–500 CFUs), or 4 (> 500 CFUs). Values differ from endometritis grade 0 to **P < 0.01 to ***P < 0.001. (C) Endometritis grade is prognostic for treatment success (Sheldon & Noakes 1998); treatment success rates were determined as the percentage of animals (n = 300) with normal vaginal mucus 2 weeks after initial endometritis grading and treatment 21 to 28 days postpartum. Values differ between endometritis grades, *P < 0.05 and **P < 0.01. (From Sheldon IM, Cronin J, Goetze L, et al. *Biology of Reproduction.* 2009;81:1025–1032.)

interval has been shown to be an average of 12 days (Tennant & Peddicord 1968), 10 days (Bretzlaff et al. 1982), and 31 days (Borsberry & Dobson 1989). Endometritis also increased services per conception from 1.67 and 2.16 to 2.0 and 2.42, respectively (Tennant & Peddicord 1968, Bretzlaff et al. 1982). In two surveys, culling for failure to conceive was 14% and 21%, respectively, in cows with metritis compared with an average of 6% or 5% for unaffected animals (Tennant & Peddicord 1968, Bretzlaff et al. 1982).

The state of the uterus and its contents has been used as a prognostic indicator for cows with endometritis. For example, a significant correlation was found between the state of the uterus, as determined by rectal palpation, and the calving–conception interval, especially in relation to the amount of pus in the discharge (Studer & Morrow 1978). Similarly, observations of the nature of purulent vaginal discharges have also been used as a prognostic indicator. The presence of a purulent discharge and cervical diameter larger than 7.5 cm was also associated with impaired fertility (LeBlanc et al. 2002a). There tended to be an increasing impairment of fertility with increasingly purulent discharge. Mucopurulent discharge tended (P = 0.09) to increase time interval to pregnancy. In another study, purulent or foul discharge was consistently associated with a 20% reduction in pregnancy rate (Sheldon & Noakes 1998).

Endometritis therefore reduces the profitability of a dairy enterprise, and the cost can be calculated by relating it to the increase in the calving–conception interval. Losses are mainly due to an extended calving–conception interval, increased culling rates, reduced milk yield, and the cost of treatment (Kossaibati & Esslemont 1997). Most of these losses are preventable by instigating effective treatment of affected animals before the end of the voluntary wait period or start of the breeding season.

Treatment

Few aspects of theriogenology have attracted more debate than the treatment of endometritis. Two problems that impede the study of its treatment are: spontaneous 'self-cure', which has been estimated to be between 33% (Steffan et al. 1984) and 46% (Griffin et al. 1974a); and the variable criteria that have been used for its diagnosis before the setting out of agreed definitions (Sheldon et al. 2006).

There is, however, consensus on the following:

- There is little merit in performing routine swabbing and bacterial sensitivity tests before treatment.

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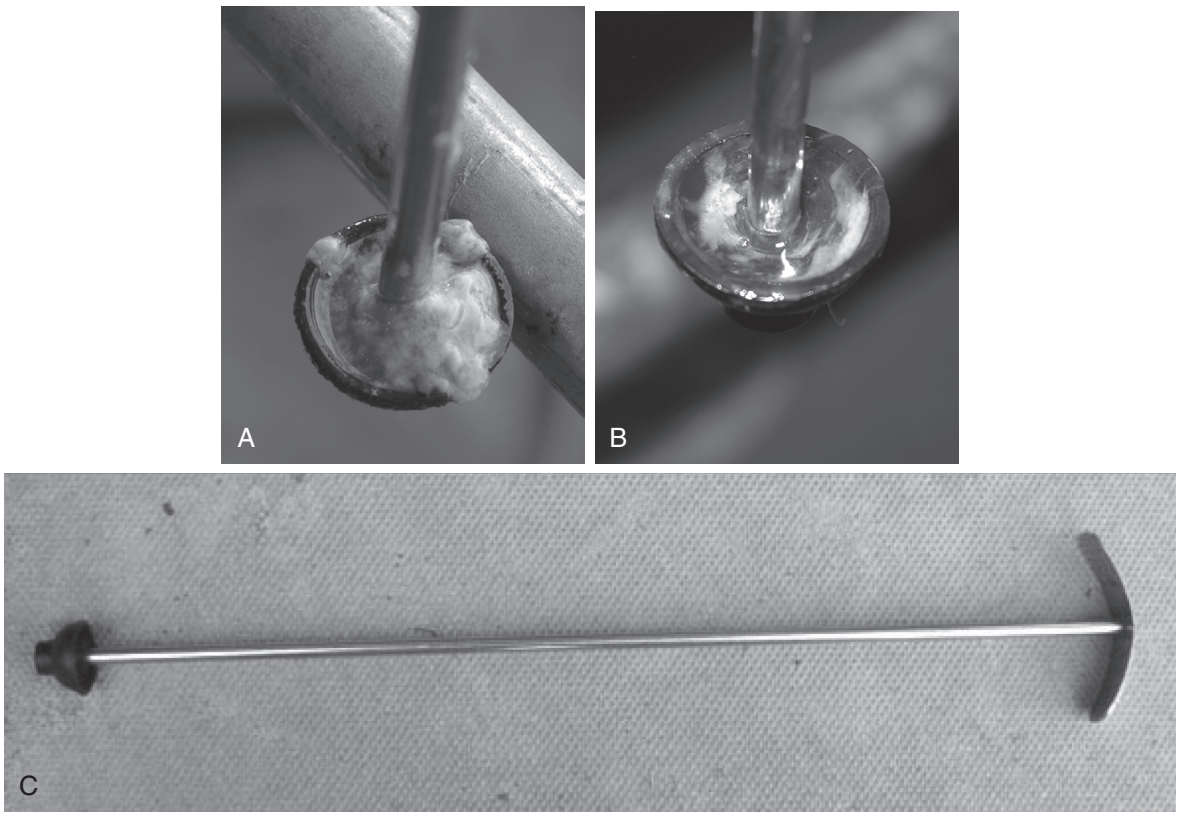

• **Fig. 23.11** The vaginal mucus can be sampled and pus (A) or mucopurulent material (B) detected using a Metricheck instrument (C).

TABLE 23.5 Factors associated with subfertility due to endometritis

Assisted calvings, especially when internal manipulation is required
Retained fetal membranes
Purulent vaginal discharges in the interval between calving and the start of mating
Premature calving (including twins, abortion); induction of calving before term has adverse effects on fertility in some, but not all studies
BUT NOT uncomplicated cases of hypocalcaemia

From Morton 2000.

- Intrauterine infusion of antiseptics is of limited value and, at worst, injurious to the uterus.
- Treatment should be delayed until at least 3 weeks postcalving (LeBlanc et al. 2002b).
- Rational therapy is based upon either hormonal stimulation of uterine defences or the use of antibiotics.

Stimulating Uterine Immunity

Three main methods have been used: (1) stimulation of oestrus with $PGF_{2\alpha}$ in animals that have an active corpus luteum (CL); (2) administration of low doses of oestradiol; and (3) stimulation of oestrus in animals that are anoestrous.

When there is a mature CL on the ovary, $PGF_{2\alpha}$ (or an analogue) is an effective means of treatment for clinical endometritis. Administration of $PGF_{2\alpha}$ causes luteolysis, thereby reducing progesterone concentrations and stimulating the return of oestrus. The cow will return to oestrus 3 to 5 days after treatment, often with a mild to moderate purulent vaginal discharge. It is preferable that animals should be treated before the end of the voluntary wait period, but if treatment has been delayed until after the start of the mating period, it is possible to serve or inseminate at the induced oestrus, unless the discharge is severe. Some cases of endometritis present with leukorrhoea when the cow is in oestrus; for these animals, $PGF_{2\alpha}$ may need to be given 7 to 10 days later when a responsive CL would be present. The response to $PGF_{2\alpha}$ treatment in terms of conception at the induced oestrus and final pregnancy rate is generally good (LeBlanc et al. 2002b) and markedly better than in untreated controls. Some have advocated intrauterine administration of $PGF_{2\alpha}$, but this appears to confer no particular advantages. Interestingly, pathogens switch prostaglandin secretion in the endometrium away from PGF towards PGE, which may also be why $PGF_{2\alpha}$ is a successful therapeutic (Herath et al. 2009a). Whether $PGF_{2\alpha}$ administration to cows without a CL is beneficial is not clear; in some cases there is a positive response but not in other cases (Steffan et al. 1984, LeBlanc et al. 2002b).

Historically, intramuscular injection of 3 to 5 mg of oestradiol benzoate to cows without a detectable CL has been used with some clinical success to treat endometritis (Sheldon & Noakes 1998), although fertility was not improved. The rationale for the use of oestrogens is that they increase uterine blood flow and stimulate the immune system, as occurs during oestrus. The withdrawal in the European Union of licensing for the use of oestrogens in food-producing animals means that this treatment is no longer legal in animals that are to supply milk or meat to those countries. High

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doses of oestrogens are contraindicated as they result in long-term down regulation of the hypothalamic–pituitary axis, resulting in anoestrus and ovarian cysts.

In animals without a CL, it is theoretically possible to induce oestrus with intravaginal progesterone-releasing inserts, but this practice is not recommended as many carry a contraindication for use in animals with vaginal or uterine infection. Induction of oestrus with gonadotrophin-releasing hormone (GnRH) has also been advocated as a means of treating endometritis in acyclic cows, although this is seldom undertaken now. With the use of ovsynch programmes for fixed time AI (see Chapters 8 and 27), many postpartum animals are inadvertently treated for endometritis as part of routine reproductive programmes in many dairy herds. However, studies suggest that ovsynch programmes have little benefit for the treatment of endometritis, and endometritis did not affect the outcome of ovsynch (Hendricks et al. 2006, Kasimanickam et al. 2006).

Antibiotics

A wide range of antimicrobial agents has been used in the treatment of endometritis. Although parenteral antibiotic treatment is needed for metritis, it is generally considered to be preferable to treat endometritis via the intrauterine route. Provided an adequate dose of antibiotic is used, this will result in effective MICs reaching the endometrium and being established in the intraluminal secretions. The latter point is important for the effective treatment of the disease because subtherapeutic dose rates are frequently used. In organic herds, antibiotics are not often permitted, and so mild antiseptics may be used, such as 2% povidone iodine or a hibitane solution (Mido et al. 2016). Some clear principles underlie the choice of antimicrobial and/or antiseptic agents:

- It must be effective against the wide range of aerobic and anaerobic, Gram-positive and Gram-negative bacteria that are present.
- It must be effective within the microaerophilic environment of the uterus.
- Whether an effective bactericidal or bacteriostatic concentration can be achieved at the site of infection by the intrauterine route of administration. When the intrauterine route is used, the substance must be evenly and rapidly distributed throughout the uterine lumen with good penetration into the deeper layers of the endometrium.
- It must not inhibit natural uterine defence mechanisms, particularly the cellular component.
- It must not traumatise the endometrium. Several of the vehicles used in the formulation of pharmaceutical preparations can damage the endometrium. Examples include propylene glycol, which can cause a necrotising endometritis; oils, which can cause granulomata; and chalky bases, which can cause irritation and blockage of endometrial glands.
- Treatment must not reduce fertility by producing irreversible changes in the reproductive system.
- Treatment must be cost effective by enhancing fertility.
- Details of its absorption from the uterus and excretion in the milk must be known, so that appropriate withdrawal times can be followed.

In consequence, several antibiotics are inappropriate. Nitrofurazone is irritant and has an adverse effect on fertility. Aminoglycosides are not effective in the predominantly anaerobic environment of the infected uterus. Field trials have also provided evidence for a lack of effectiveness of these drugs in the treatment of endometritis. Sulphonamides are ineffective because of the presence of paraaminobenzoic acid metabolites in the lumen of the infected uterus. Penicillins are susceptible to degradation by the large numbers of penicillinase-producing bacteria that are present in the diseased uterus.

Antibiotics that have been formulated for intrauterine use include oxytetracycline as an intrauterine infusion or in the form of pessaries and cephapirin as an infusion. Parenteral oxytetracycline penetrates the uterine wall and lumen (Ayliffe & Noakes 1978), but the MIC for many organisms is higher than can be achieved practically (Sheldon et al. 2010). Intrauterine oxytetracycline does not penetrate the wall of the uterus well, is not particularly effective against *T. pyogenes,* and can have a direct irritant effect upon the endometrium (Cohen et al. 1995). The main role for tetracycline is therefore as a prophylactic intrauterine antibiotic after assisted calvings. However, cephapirin has been widely studied as an intrauterine treatment for endometritis with positive results (LeBlanc et al. 2002b, Kasimanickam et al. 2005).

Prostaglandin $F_{2\alpha}$ Versus Intrauterine Antibiotics

A comparison was made of the effectiveness of intrauterine infusion of 1500 mg oxytetracycline hydrochloride with intramuscular injection of the $PGF_{2\alpha}$ analogue cloprostenol or 3 mg estradiol benzoate as treatments for endometritis (Sheldon & Noakes 1998). It was concluded that, provided a CL was present, $PGF_{2\alpha}$ was the most successful treatment, both in terms of cure rate and calving to conception interval (Sheldon & Noakes 1998). Oxytetracycline was more effective than oestradiol but marginally less so than $PGF_{2\alpha}$. Similar results were found comparing a commercial antibiotic preparation with $PGF_{2\alpha}$ and oestradiol (Pepper & Dobson 1987). In another study the effect of intrauterine cephapirin on fertility of cows with endometritis was better than for a prostaglandin analogue if animals were unselected for the presence of a CL; but when cows with a CL were treated with PGF, the results of the cephapirin and prostaglandin treatments were similar (LeBlanc et al. 2002b). In the majority of the foregoing treatments the severity of the case adversely affected the cure rate. Thus the severity of endometritis should be recorded at the time of treatment and should be used for selecting animals for reexamination, and for prognosis (see Fig. 23.10C).

Subclinical Endometritis

Uterine biopsy has been used to study both the incidence of clinical and subclinical endometritis. Biopsies can be collected using a range of specialised instruments for endometrial biopsy, such as that shown in Fig. 23.12, or by using balloon biopsy forceps. Uterine biopsies collected from infertile cows reveals that many have subclinical endometritis. For example, a study in 1971 reported that 77% of infertile cows had endometritis: bacterial infection was found in 64% of these cows and 80% had lesions of endometritis evident in biopsies (Sagartz & Hardenbrook 1971). Likewise, another study showed that 50% of the genital tracts obtained from an abattoir showed histological evidence of endometritis, yet only 12.5% showed gross lesions (Hartigan et al. 1972). Hence the evidence from such biopsy studies is that many cases of endometritis are subclinical with no purulent discharge evident.

Although histological analysis of endometrial biopsies had previously shown endometrial inflammation in the absence of clinical signs, the importance of subclinical endometritis was first demonstrated in field studies by collecting samples from the surface of the endometrium using a cytobrush or uterine lavage (Kasimanickam et al. 2004, Gilbert et al. 2005). These studies found that the presence of polymorphonuclear neutrophils (PMNs) in smears

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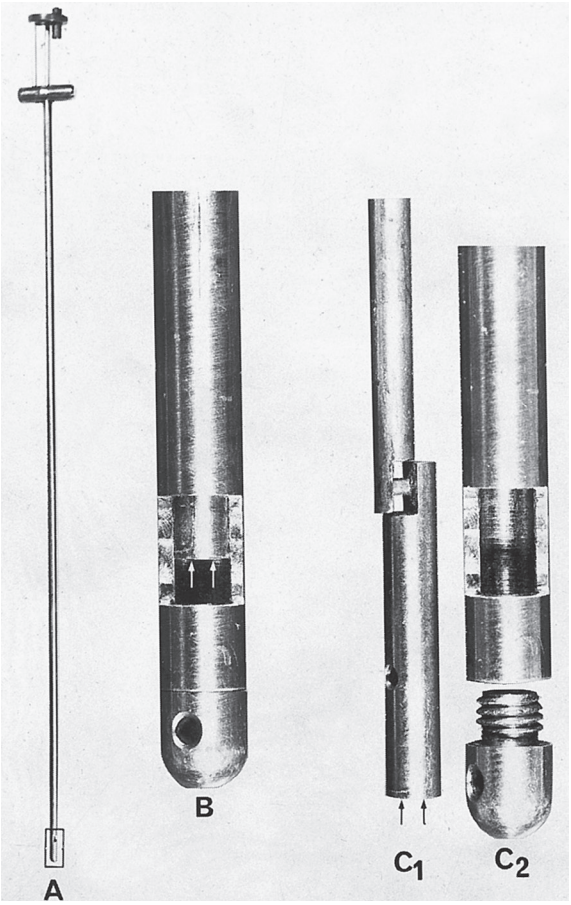

• **Fig. 23.12** Uterine biopsy instrument (Hartigan et al. 1974). (A) Whole instrument showing window with cutting edge. (B) Close-up with edge partially withdrawn (*arrow*). Close-up views (C1, C2) show the cutting edge (*arrow*) and interchangeable tip (C2).

made from the endometrial samples was associated with reduced pregnancy rates. Consequently, subclinical disease is defined by the proportion of PMNs exceeding operator-defined thresholds, usually about 5% of cells in samples collected by flushing the uterine lumen or by endometrial cytobrush, in the absence of clinical endometritis, about 35 to 40 days postpartum (Sheldon et al. 2006, de Boer et al. 2014). In addition, if the cytology samples are analysed for gene expression, there is typically increased expression of inflammatory mediators (Gabler et al. 2009, Fischer et al. 2010, Gabler et al. 2010, Ghasemi et al. 2012).

Diagnosis

The cytobrush technique typically uses a cytobrush used for human cervical cytology that is screwed into a metal rod (Fig. 23.13), which is then protected by a plastic sheath, and inserted through the cervix using a method similar to artificial insemination. The brush is extruded and rolled along the endometrium in the body or horns of the uterus (Bogado Pascottini et al. 2016). After removing the instrument from the animal, a cytology smear is prepared on a glass slide. Alternatively, the uterine flush technique uses approximately 20 mL of sterile saline infused into the uterus through a catheter; the uterus is massaged, and the fluid aspirated via the same catheter (Gilbert et al. 2005). The suspension is then used to prepare a cytology smear on a glass slide, usually using a specialised centrifuge instrument designed to deposit cells evenly onto a glass slide, such as a cytospin machine. The slides are stained, and using a light microscope, the glass slides are examined, with 100 to 400 cells counted and the proportion of PMNs recorded. It is now suggested that counting 300 cells provides optimal reproducibility among observers (Melcher et al. 2014).

There is much debate about the proportion of PMNs that indicate the presence of subclinical endometritis, and the criteria differ with the time after parturition and vary among farms and operators (Sheldon et al. 2006, Melcher et al. 2014, Wagener et al. 2017). Criteria for the diagnosis of subclinical endometritis were proposed for different stages postpartum based upon the proportion of neutrophils present in cytology samples from cervical swabs (Table 23.6). For large herds it is possible to generate a farm-specific cut-off point for diagnosis of subclinical endometritis that compromises fertility. However, beyond 50 days postpartum the presence of any PMNs in cytology samples is now often regarded as abnormal. Interestingly, cytology does not always correlate well with endometrial biopsy (Westermann et al. 2010, Bogado Pascottini et al. 2016). However, both techniques are not practical for commercial dairy farms, and so alternatives are being sought to provide a cow-side test.

There remain many open questions about subclinical endometritis. This may partly be because inflammation is part of the normal repair process of the tissues during the puerperium, as well as infection also stimulating the accumulation of neutrophils in the uterus. The inflammation may also be associated with recovery of the tissues after metritis and clinical endometritis, trauma, or other non-microbial disease. In one study of 528 animals, more animals with metritis subsequently showed evidence of subclinical endometritis than animals without metritis (Lima et al. 2014). However, it is not clear whether subclinical endometritis is absolutely dependent on the presence of pathogens or may reflect dysregulation of the innate immune response even in the absence of pathogens.

Treatment

Current ideas for treating subclinical endometritis were reviewed recently (Wagener et al. 2017). Unfortunately, there is little agreement about whether or how animals with subclinical endometritis should be treated. On the basis that physiological mechanisms usually aim to restore homoeostasis, the most rational treatment for subclinical endometritis is to administer $PGF_{2\alpha}$ (or analogue) to animals with a CL. Other suggestions are the intrauterine infusion of antimicrobials or antiseptics or to flush the uterus as if performing an embryo collection.

Pyometra

Pyometra is defined as a progressive accumulation of purulent material within the uterus in the presence of an active CL (Sheldon et al. 2006). The condition occurs most commonly when uterine infection is not eliminated during the first follicular phase, and the resulting inflammation means that the uterus ceases to produce PGF. Consequently, the life span of the CL is prolonged. In most cases, pyometra occurs as a sequel to endometritis during the postpartum period. Venereal infections that cause embryonic death (notably *T. fetus*) can predispose to a high incidence of pyometra in an infected herd after mating, although occasional cases also occur after fetal death, maceration, and superinfection with *T. pyogenes.*

Diagnosis

Cows that suffer from pyometra show few or no signs of ill health. Hence the disease is generally discovered**:** (1) when affected cows

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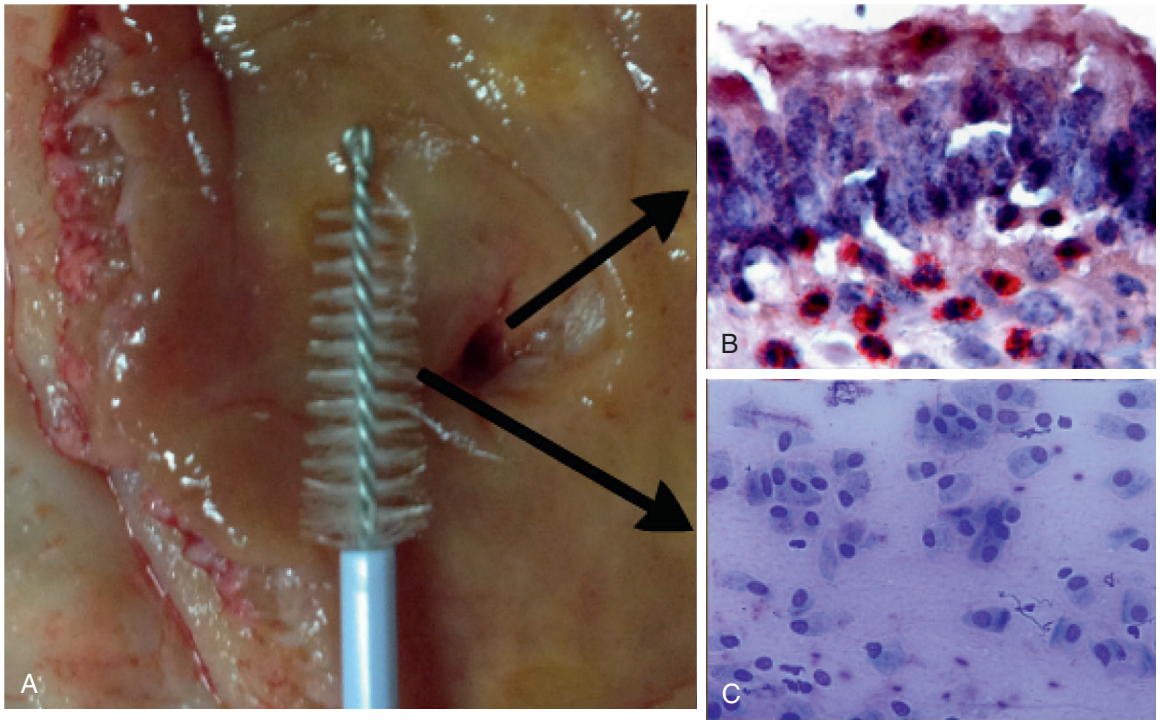

• **Fig. 23.13** Subclinical endometritis evaluation (Bogado Pascottini et al. 2016). (A) Image showing how the endometrial biopsies and cytology samples were taken next to each other with an 8.0 mm punch biopsy and a cytobrush, respectively. (B) Endometrial histopathology sample stained with Naphthol-AS-D-chloroacetate-esterase. (C) Endometrial cytology sample stained with Wright-Giemsa. (From Bogado Pascottini O, Hostens M, Dini P, et al., *Theriogenology* 2016;86,1550–1556.)

TABLE 23.6 Criteria for the diagnosis of subclinical endometritis from cervical swabs

Days After Calving	% PMN in Cytology Samples
20–33	> 18
34–49	> 10
> 50	> 5

PMN, polymorphonuclear leucocytes.
From Sheldon et al. 2006.

are examined for the absence of cyclical activity; or (2) at the time of pregnancy diagnosis in a cow that was thought, on the basis of non-return to oestrus, to be pregnant. Because the cervix remains closed, the purulent exudate accumulates within the uterine lumen, although occasionally there is a slight or intermittent purulent discharge. The uterine horns are enlarged and distended (Fig. 23.14), quite often to an unequal degree, owing to incomplete involution of the previously gravid horn or to recent conceptus death. Differentiation of pyometra from a normal pregnancy can sometimes be difficult, but there are a number of distinguishing points:

- The uterine wall is thicker than at pregnancy.
- The uterus has a more 'doughy' and less vibrant feel.
- It is not possible to slip the allantochorion (see Chapter 5).
- In some cases of pyometra, no uterine caruncles can be palpated. However, when the infection occurred in a non-involuted uterus, involution of the caruncles is delayed, and they may remain palpable for quite a long time.
- Transrectal ultrasonography will demonstrate the absence of a fetus and the presence of a speckled echotexture of the uterine contents compared with the black anechoic appearance of normal fetal fluids.

If there is any doubt about the differentiation between pyometra and pregnancy, the cow should be left untreated and reexamined later for evidence of change. Pyometra associated with *T. fetus* infection presents features that are different from those previously described. Uterine pus is, as a rule, much more copious and may attain a volume of many litres, and the uterus undergoes much greater distension than postpartum pyometra; the uterine pus is often fluid and is greyish-white or white. The mucus occupying the cervix is moist and slippery rather than sticky and tenacious, and motile trichomonads can generally be found in it.

Treatment

When the presence of pyometra is discovered at the time of pregnancy diagnosis, the decision is often made to cull the cow at the end of the current lactation. Treatment, if undertaken, is with $PGF_{2\alpha}$, which results in regression of the CL, dilation of the cervix, expulsion of the purulent fluid, and oestrus 3 to 5 days later. Provided that the condition is not too long standing and therapy is instituted quickly, there is a reasonable possibility that the cow will eventually conceive again. However, long-standing cases are associated with more severe degeneration of the endometrium, reducing the chances of subsequent conception. There may be advantages to the use of intrauterine cephapirin in conjunction with $PGF_{2\alpha}$ (or analogue), and multiple treatments at 11 to 14 day intervals may salvage animals for future breeding.

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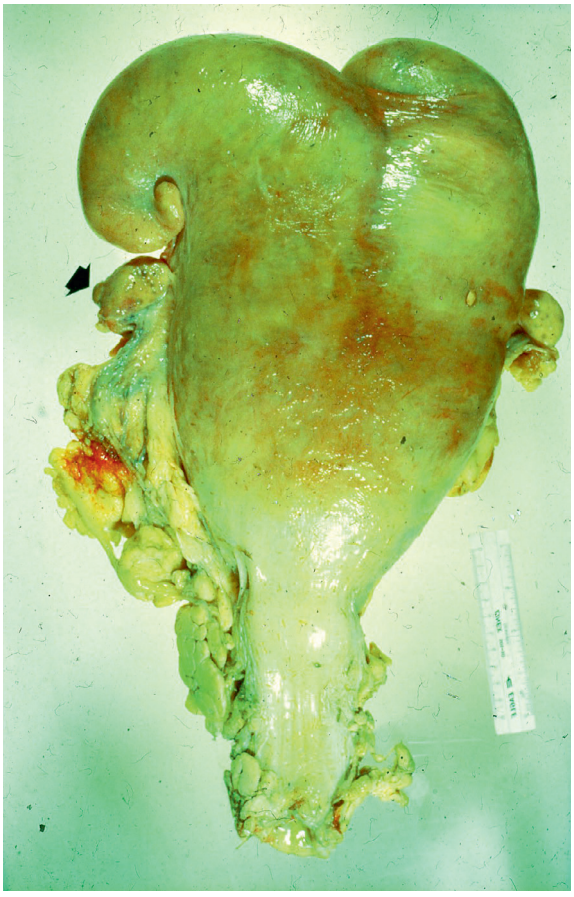

• **Fig. 23.14** Uterus with pyometra, with evidence of a CL (*arrow*) with distended uterine horns.

Cervicitis and Vaginitis

Cervicitis and vaginitis may be caused by trauma during parturition or during obstetrical manipulation. Tearing of the cervix, vagina, and vulva are often self-evident during and after parturition. If these tears become infected, they can cause considerable swelling and pain, and animals often strain frequently. Treatment for vaginitis is usually symptomatic with emollient creams applied to the injured tissues and parenteral and/or local antibiotics.

Purulent vaginal discharge 21 days or more postpartum may indicate cervicitis or vaginitis rather than clinical endometritis. In some cases, purulent vaginal discharge may be associated with both vaginitis and endometritis; in other cases purulent vaginal discharge may represent vaginitis or cervicitis rather than endometritis (Denis-Robichaud & Dubuc 2015). It is thought that cervicitis, 21 days or more postpartum, is associated most often with prior obstetrical trauma and/or RFM. Cervicitis was diagnosed in 61% of animals in a study of 416 cows examined at 42 to 50 days postpartum (Hartmann et al. 2016). Cervicitis can affect fertility, with fewer animals pregnant by 300 days in milk (Deguillaume et al. 2012). Treatment of purulent vaginal discharge that is caused by cervicitis alone is often symptomatic, and so most of these animals are treated as if they had clinical endometritis.

Effect of Uterine Disease on Ovarian Function

Pathological changes in the endometrium during uterine disease are likely to be detrimental to fertilisation and conception. However, postpartum uterine infections also impair fertility after resolution of the clinical disease (Borsberry & Dobson 1989). Thus one must consider if uterine infections impair the oviduct, ovary, or the hypothalamic–pituitary axis.

The uterine tubes (oviducts) impede bacteria reaching the ovary; consequently, ovarian infections or abscesses are rare. However, extension of infection or inflammation into the oviduct likely disrupts the delicate balance of the immune systems that are required for fertilisation (Marey et al. 2016).

Beyond the tubular genital tract, uterine infection may impair ovarian function (Sheldon et al. 2014a, Bromfield et al. 2015). At a practical level, veterinarians should be aware that individual dairy cows with postpartum uterine infections have a slower growth of the dominant follicle, lower peripheral plasma oestradiol concentrations, and are less likely to ovulate (Sheldon et al. 2002b). At the herd level a history of uterine infection or dystocia are associated with a delayed return to ovarian cyclic activity, more cystic ovarian disease, and with prolonged luteal phases (Opsomer et al. 2000).

One possible mechanism linking uterine disease to ovarian dysfunction is that cytokines and PAMPs perturb the endocrine function of the hypothalamus and the pituitary, reducing the release of GnRH and LH, which effects ovarian function (Peter et al. 1989, Karsch et al. 2002). In postpartum cows, endotoxin in the uterus suppresses the luteinising hormone (LH) surge and prevents ovulation (Peter et al. 1989). In addition, during the follicular phase, endotoxin decreases LH pulse frequency, decreases oestradiol, and interrupts the LH surge and ovulation in cattle (Suzuki et al. 2001, Lavon et al. 2008). Similar observations have been made in sheep, which is the species most often used to study the effect of endotoxin in the ruminant brain (Karsch et al. 2002). Neuroendocrine activity at the hypothalamic level is inhibited by endotoxin suppressing both the frequency and amplitude of GnRH/LH pulses. However, there are also effects within the pituitary gland, as endotoxin lowers LH responses to exogenous GnRH (Karsch et al. 2002). Indeed, treatment of ewes with endotoxin suppresses the expression of genes encoding GnRH receptor (GnRHR) and LHβ (Herman et al. 2013). However, the peripheral plasma concentrations of FSH are unaffected by uterine disease in cattle, so that recurrent waves of follicles develop in the ovary, even during uterine disease (Sheldon et al. 2002b).

A second mechanism is that the cytokines, associated with the host defence response to bacteria in the uterus, may reach the ovary via the localised countercurrent mechanism, as used by prostaglandin $F_{2\alpha}$ during luteolysis (see Chapter 1). For example, cytokines such as IL-6 and TNFα perturb bovine ovarian follicular cell steroidogenesis (Alpizar & Spicer 1994, Spicer 1998).

A third mechanism linking infection of the endometrium with ovarian function is that PAMPs might also reach the ovary from the uterus. The concentrations of endotoxin in follicular fluid aspirated from dominant follicles is correlated with the severity of uterine disease (Herath et al. 2007). Notably, healthy ovarian follicles do not contain hematopoietic immune cells, so ovarian follicle responses to cytokines or PAMPs must rely on the granulosa cells and oocyte (Herath et al. 2007, Bromfield & Sheldon 2011). Indeed, endotoxin limits granulosa cell oestradiol production by reducing *CYP19A1* gene expression and aromatase protein levels (Herath et al. 2007, Price et al. 2013). Interestingly, granulosa cells isolated from growing or dominant ovarian follicles express most of the TLRs (Bromfield & Sheldon 2011, Price et al. 2013). Furthermore, PAMPs, such as bacterial endotoxin or bacterial lipopeptides, stimulate an inflammatory response by granulosa cells, with the secretion of IL-1β, IL-6, CXCL1, CXCL2, CXCL3,

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and IL-8 protein (Bromfield & Sheldon 2011, Price et al. 2013). Inhibiting *TLR4* or *TLR2* gene expression in bovine granulosa cells using siRNA reduced the secretion of IL-6 in response to their cognate PAMPs (Bromfield & Sheldon 2011, Price et al. 2013). So, granulosa cells in antral follicles clearly have roles in innate immunity. Endotoxin also reduces the primordial ovarian follicle pool with an associated increase in primordial follicle activation and loss of primordial follicle regulatory proteins (Bromfield & Sheldon 2013). However, not all stages of follicle development are sensitive to PAMPs, and endotoxin did not affect the growth and viability of individually cultured secondary follicles (Bromfield & Sheldon 2013).

During the later stages of ovarian follicle development, endotoxin stimulates IL-6 secretion from cumulus-oocyte complexes and activates cumulus expansion *in vitro* (Bromfield & Sheldon 2011). Inappropriate timing of cumulus expansion may contribute to infertility because expansion is normally closely coordinated with ovulation. Furthermore, endotoxin or IL-6 might reach the oocyte via the cytoplasmic transzonal projections from granulosa cells that synapse on the oolema. Indeed, endotoxin increases the incidence of meiotic arrest and germinal vesicle breakdown failure in bovine oocytes (Bromfield & Sheldon 2011). Furthermore, treatment of cumulus-oocyte complexes with endotoxin or PAM perturbed expression of genes such as *GDF9* and *NLRP5,* which are involved in oocyte maturation (Sheldon et al. 2014b). Oocyte development takes about 120 days between the primordial follicle stage to ovulation of a cumulus-oocyte complex. Thus it has been suggested that in cows inseminated 60 to 120 days postpartum, the oocytes that are ovulated may have been perturbed and be less fertile after exposure to metabolic stress (Britt 1992). Therefore limiting uterine disease is not only important for the affected animals but also for their offspring.

Conclusions and Outlook

Although there is a clear understanding of the clinical aspects and implication of postpartum uterine disease and some of the mechanisms of pathology, there are important outstanding questions. The most obvious question is: why are modern high-milk-yield cows so susceptible to metritis and endometritis? Allied to this, is what can be done to prevent uterine disease? Answering these questions is vital for sustainable intensification of the dairy industry over the next 50 years. Genetic selection for more resilient animals that are less susceptible to uterine disease is a priority for the dairy industry. In the meantime, veterinarians should consider that prevention is better than cure, and advise clients about animal nutrition and management to increase the resilience of parturient and postpartum animals so that they are better able to tolerate the presence of uterine pathogens without developing uterine disease.

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24

Specific Infectious Diseases Causing Infertility and Subfertility in Cattle

TIMOTHY J. PARKINSON

Many of the infectious diseases of cattle adversely affect reproductive performance, either by direct effects upon the reproductive system or via indirect effects upon the general state of health of affected animals. In this chapter the effects of enzootic infectious diseases upon reproductive performance are considered; the effects of non-specific infections of the reproductive tract, such as the metritis complex, were considered in Chapter 23.

Infectious diseases can affect the reproductive system through:

- Impaired sperm survival or transport in the female tract, leading to reduced fertilisation rate;
- Direct effects upon the embryo, including infections that result in early embryonic death and those that infect the more advanced fetus or its placenta, resulting in abortion, stillbirths, or the birth of weak calves; and
- Indirect effects upon embryo survival, including infections that have adverse effects upon uterine function and those that infect the maternal component of the placenta. Again, these result in embryonic death, fetal death with abortion, mummification, or stillbirth.

The patterns of enzootic infectious diseases that affect reproduction have changed considerably in most developed countries over the past 40 to 50 years. The classic venereal diseases, campylobacteriosis and trichomonosis, have been largely eradicated in dairy cattle by the use of artificial insemination (AI) with semen from disease-free bulls. In beef cattle, in which natural service remains the predominant method of breeding, the control has been less complete. Most Western countries have successfully eradicated brucellosis through programmes based upon vaccination, blood testing, and slaughter. Conversely, other diseases, such as infectious bovine rhinotracheitis/infectious pustular vulvovaginitis (IBR-IPV), bovine viral diarrhoea (BVD), and leptospirosis, have assumed much greater importance, either because of a genuine increase in prevalence or the development of better diagnostic methods. Other diseases, whose effects upon reproduction were hitherto unrecognised, are now ascribed significance as reproductive diseases. Examples include ureaplasmosis, *Histophilus somni* infections, and abortions due to *Neospora caninum.* Yet, even though there has been a change in the importance of different specific infectious agents in causing infertility, none should be forgotten when investigating subfertility in a herd. Diseases that have been considered as being eliminated can still reemerge and can cause catastrophic effects if they gain entry to a herd with a low immune status to that disease.

Estimates of the prevalence of infectious diseases of reproduction largely depend on the successful diagnosis of causes of abortion. The data provided from this source provide only an approximate guide to the prevalence of diseases, however, because the percentage of fetopathies from which a specific infectious agent is identified is relatively small. In the results from the Veterinary Laboratories Agency of the Department for Environment, Food and Rural Affairs (UK), positive results were obtained in approximately 10% to 14% of submissions. When statutory submissions for brucellosis screening are omitted, the diagnostic rate is approximately 20% to 30%. Nonetheless, such data do show that the prevalence of many infectious causes of abortion has been relatively static or declining in the UK (Table 24.1) since first publication of the Veterinary Investigation Diagnosis Analysis (VIDA II) in 1977 (Veterinary Laboratories Agency 2016).

Bacterial Agents

Bovine Venereal Campylobacteriosis

Campylobacteriosis was spread around the major livestock-producing regions of the world with the early importations of cattle. Today, venereal campylobacteriosis is still one of the most important infectious causes of infertility in cattle throughout the world, despite prolonged efforts to eradicate it. Wherever the main method of breeding is by natural service (notably in beef herds), its venereal route of transmission has ensured that the organism remains a threat to cattle fertility. Most beef-producing countries are affected, including the US, Canada, Australia, Argentina and other countries in South America, and several countries in Africa (Michi et al. 2016). The disease occurs either with a continuous epizootic presence or with reemergence of the condition after a long period of supposed absence (e.g., the UK; MacLaren & White 1977). Where AI is the main method of cattle breeding (notably in dairy cattle), the incidence of campylobacteriosis has declined significantly.

Aetiology

Campylobacter fetus (originally named *Vibrio foetus*) is divided into two main subspecies. The most important of these in terms of causing bovine infertility is the subspecies *venerealis* (CFV), which, as its name suggests, is spread through coitus. The subspecies *fetus* (CFF, of which there are two main serotypes) is not spread venereally and is not normally considered to be a major cause of infertility

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TABLE 24.1 Frequency (n) of isolation of pathogens from bovine fetopathies examined by the Veterinary Laboratories Agency of the Department for Environment, Food, and Rural Affairs (UK)

	2007	2009	2011	2013	2015
Bacillus licheniformis	188	114	117	82	69
Brucella abortus	30	1	0	0	0
Campylobacter spp.	27	37	34	22	21
Leptospira spp.	54	5	1	2	2
Listeria monocytogenes	32	33	24	38	22
Salmonella Dublin	38	75	72	79	60
Salmonella Typhimurium	1	1	2	1	0
Other *Salmonella* serotypes	6	12	10	5	
Trueperella pyogenes	56	95	96	58	60
Bovine herpesvirus-1 (IBR-IPV)	3	5	5	6	1
Bovine viral diarrhoea	63	44	24	25	36
Schmallenberg virus	0	0	0	44	0
Neospora caninum	320	149	114	100	104
Fungi	49	64	56	36	33

From: https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/541560/vida-cattle-08-15.pdf.

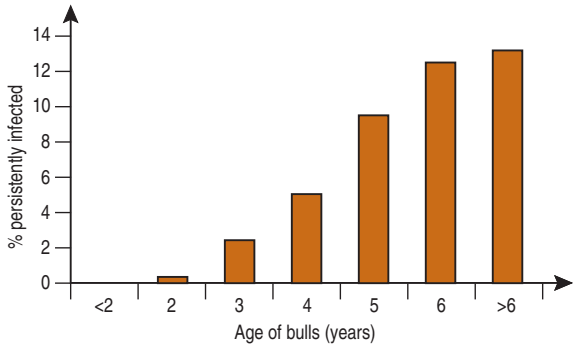
in cattle, although it can cause sporadic abortions (Thompson & Blaser 2000). In addition, 'intermediate' strains of CFF have occasionally been reported as a cause of infertility akin to that produced by CFV (MacLaren & Agumbah 1988). There have been many changes to the names given to the subspecies of *C. fetus* over the years, which make it difficult to follow the early literature.

Other species of *Campylobacter (C. coli, C. hyointestinalis, C. jejuni,* and *C. sputorum*) have been implicated in bovine abortions (Newell et al. 2000). In addition, there are a number of saprophytic species of *Campylobacter*, which may be present in the alimentary tract of cattle or in the prepuce of the bull. The main significance of these latter organisms (in terms of venereal campylobacteriosis) is that they can interfere with the diagnosis of pathogenical species.

Infection in the Bull

Infection of the bull does not cause any visible lesions of the genital tract nor does it cause interference to its reproductive behaviour or its semen quality. The bull simply acts as a carrier and transmits the infection at service to the female. The primary route of infection is venereal transmission. There are, however, also reports that bull-to-bull transmission may occur through mounting when bulls are confined together (Wagner et al. 1965). Infection through contaminated semen collection equipment is a well–recognised variant of the venereal route of transmission.

The organism is confined to the penile integument, glans penis, prepuce, and distal urethra (Thompson & Blaser 2000). Because the organism lives in the crypts of the penile integument, the likelihood of bulls becoming persistently infected increases with



• **Fig. 24.1** Relative risk of bulls of different ages becoming persistently infected with *Campylobacter fetus* subsp. *venerealis*.

age as the crypts become deeper and more extensive (Kennedy & Miller 1993). It is relatively unusual for bulls less than 4 years old to become persistently infected (Philpott 1968). Early work showed that only 5% of bulls under 4 years old persistently carry the infection (Adler 1956). When exposed to natural or artificial infection, few bulls less than 4 years old remained infected for no more than a few days, whereas bulls more than 4 years old generally became persistently infected (Dufty et al. 1975). Among older bulls there is less change in susceptibility with age: a study of bulls between 41 and 74 months of age (Bier et al. 1977) showed no increase in susceptibility over that period. Once infection is established, it does not usually undergo spontaneous remission: the bull remains infected for life. Typical patterns of persistent infection are shown in Fig. 24.1.

Infection in the Cow

Between 40% and 75% of non-immune female cattle become infected after a single service by an infected bull (Clarke 1971). The organism initially colonises the vagina, occasionally producing visible mild, mucopurulent vulval discharge. The organism multiplies in the vagina and, within a week, spreads to the uterus (Dekeyser 1984). Infection remains confined to the vagina in 10% to 20% of infected animals (Clarke 1971).

The organism causes a mild, subacute, mucopurulent endometritis, in which there is a substantial periglandular accumulation of lymphocytes (Dekeyser 1984). The severity of the endometritis is maximal somewhere between the 8th and 13th week after the initial infection (Estes et al. 1966, Dekeyser 1984). There is also infection of the uterine tubes in about 25% of animals, sometimes causing salpingitis (Roberts 1986). Inflammation of the cervix may also occur, causing an increased secretion of mucus. This mucus can be mixed with the uterine exudate to form a mucoflocculent vulval discharge. Infection is eliminated from the uterus and uterine tubes in a period of weeks or months, as an immune response is stimulated (Corbeil et al. 1974). Infection with CFV does not interfere with the process of fertilisation, but the low grade endometritis results in a uterine environment that is incompatible with embryonic survival (Dekeyser 1986). Thus, fertilisation occurs in the majority of susceptible females that are served by an infected bull but, because the uterine environment is unable to sustain a pregnancy as a consequence of the inflammation, the embryo dies at a variable time after conception.

Immunity (immunoglobulin (Ig)G-based) develops relatively slowly within the uterus, but when it has done so and the infection is eliminated from the uterus, cows conceive and remain pregnant (Thompson & Blaser 2000). Hence, after an average of about five

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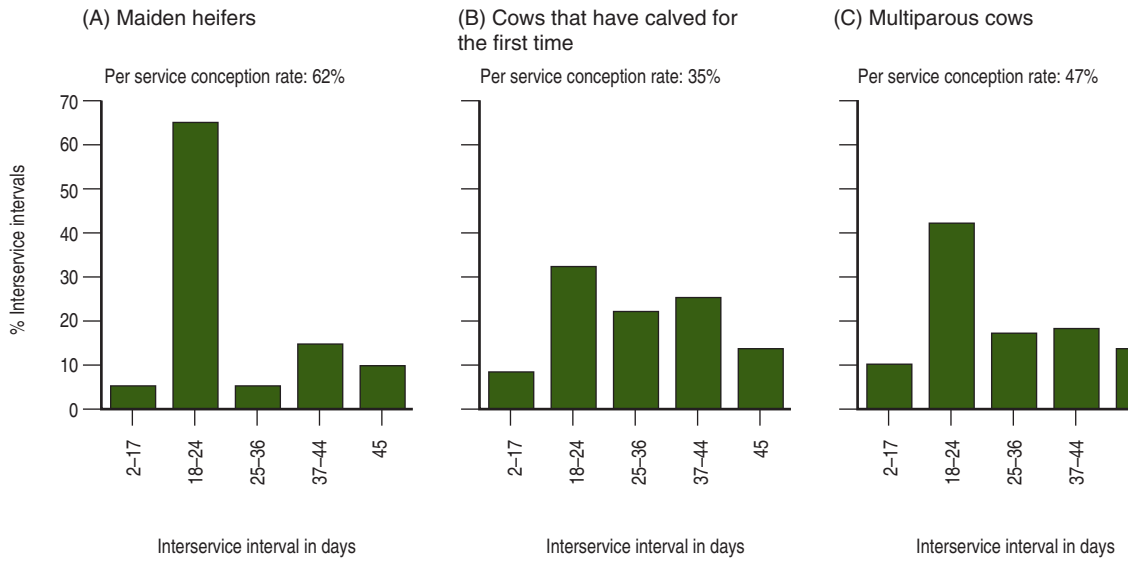

• **Fig. 24.2** Distribution of interservice intervals in a dairy herd in which there is enzootic infection with *Campylobacter fetus* subsp *venerealis.* (A) Interservice intervals were normal in maiden heifers that were served by a newly acquired (virgin) bull. (B) In first-calving cows, interservice intervals showed evidence of embryonic death, which was accompanied by poor conception rates. (C) In the older cows, interservice intervals and conception rates were improved, although chronic campylobacteriosis remains evident.

services, the majority of cows become pregnant and carry their calves to term – although a proportion of susceptible cows and heifers conceive to first service by an infected bull and carry their calves to full term. About 5% of cows appear to be resistant to infection, remaining uninfected despite repeated introduction of the organism to the reproductive tract (Dufty & Vaughan 1993).

Infection can persist in the vagina for a considerable period of time as vaginal immunity is less effective than that within the uterus. The development of the persistent vaginal carrier is partly due result of the limited ability of the IgA that is produced in vaginal secretions to promote opsonization and phagocytosis. It is also due to the ability of CFV to alter its expression of surface antigens (surface layer proteins) in response to the presence of host antibodies (Garcia et al. 1995). It appears that this antigenic shift may be the primary means by which the organism escapes the hosts' immune system (de Vargas et al. 2002) in field infections. Vaginal infection persists in most cows for up to 6 months, and in approximately 50% of cows up to 10 months after the initial challenge (Vandeplassche et al. 1963, Plastridge et al. 1964). Although most (> 95%) cows manage to eliminate the infection from the vagina by the end of a normal gestation, some remain infected after calving and occasional animals (1%–2%) remain permanent vaginal carriers (Clarke 1971, Dekeyser 1984).

Clinical Presentation

The presence of venereal disease (although not specifically campylobacteriosis) should be suspected when there are high empty rates and/or a high proportion of late conceptions in natural-service herds that cannot be explained in terms of failures of other aspects of management.

When CFV infection is introduced into a naïve herd, a dramatic decrease in conception rate occurs. Per-service conception rates can be as low as approximately 20%. Infected cows return to oestrus, typically at an interval that is greater than the normal 18 to 24 day interval, depending on the time at which the embryo dies. Embryonic deaths occurring after recognition of pregnancy result in later returns to oestrus, which occur irregularly at more than 25 days after service (Fig. 24.2). The number of animals that eventually conceive depends on the length of the breeding season; usually there are several returns to service before sufficient immunity develops for the maintenance of a pregnancy. Most animals eventually become pregnant if the breeding season is long enough. A small number of animals abort, typically at 2 to 4 months of gestation (Frank et al. 1964), although neither a large number of abortions nor abortion storms are characteristic of venereal campylobacteriosis.

In a herd in which the disease is established, there are different patterns of reproductive performance in naïve and previously infected animals. In previously infected, recovered cows, vaginal infection is readily reestablished in 30% to 70% of animals that are served by an infected bull (Clarke 1971). Such infection does not establish well within the uterus, so the cows' fertility is much less impaired than during the initial episode of disease. Nevertheless, fertility never returns to normal: overall conception rates are lower than in uninfected cows, some animals abort (Roberts 1986), and occasional animals are rendered sterile through salpingitis (Clarke 1971). Hum (1996) estimated that pregnancy rates in a newly infected, seasonally calving, beef herd fall to about 40%, recovering to between 60% and 75% in a chronically infected herd. These figures compare with a 90% pregnancy rate in an uninfected herd. Naïve animals (typically either maiden heifers or first-calf heifers, depending on the herds' breeding policy) will continue to exhibit grossly impaired reproductive performance each year when they are first exposed to infected bulls.

The first sign of genital campylobacteriosis is a marked increase in the number of females returning to oestrus, some regularly and some irregularly, after service by a newly introduced bull. Although returns are not observed in many beef herds, the first sign seen by a herd manager is that often there is a disastrously low proportion of cows pregnant at the end of the breeding season. After experiencing serious infertility for about 6 months, a herd gradually becomes immune, and most of the cows that manage to conceive will be free of infection after they have calved. If infected bulls remain with the herd, reinfection of some cows when they are rebred results

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• **Fig. 24.3** Device for collecting preputial scrapings from bulls for the diagnosis of venereal campylobacteriosis or trichomonosis.

in a similar, but much less severe, infertility problem. Eventually, after 2 to 3 years, the fertility of such cows becomes acceptable, with only moderate infertility occurring (Roberts 1986). Among incoming heifers and in any newly acquired cows (i.e., in those animals that are not immune to the disease), campylobacteriosis will be perpetuated. When maiden heifers are bred to an infected bull, they will exhibit the low conception rates and irregular returns to oestrus that are characteristic of campylobacteriosis. If, however, they are bred to a virgin bull, they will not contract the disease until after their first calving, so will have normal fertility in their first season and very poor fertility in their second. Purchased animals, likewise, show the most significant effects of the disease during their first season in the herd.

Bulls are asymptomatic carriers of the infection.

Diagnosis

Methods that have been used to diagnose CFV infection include:

- Culture;
- Serology, including immunofluorescence, vaginal mucus agglutination, or vaginal mucus enzyme-linked immunosorbent assay (ELISA) tests; and
- Polymerase chain reaction (PCR).

Culture

Campylobacter fetus is a difficult organism to grow in culture. It is microaerophilic, fragile, fastidious in its media requirements, and generally obtained from an environment that is heavily contaminated with other bacteria. Consequently, although positive culture results are diagnostic of the infection, negative results must be interpreted with considerable caution. Samples that can be collected for culture include the following:

Vaginal wash: This method is a possibility, but isolation rates from females are extremely disappointing unless the animal is very recently infected. Pregnant animals are exceedingly unlikely to yield positive results; non-pregnant animals are only a little more hopeful.

Preputial scraping or the preputial wash: Swabs are much less effective than either of these methods. Bulls are much more likely to yield positive results than cows. A device for collecting preputial scrapings is illustrated in Fig. 24.3.

Samples from abortus material: Many of the isolations of CFV have been made from aborted material from cows, mainly from stomach contents of aborted fetuses. Despite the problems implicit with mustering of animals in hill country, specific effort needs to be devoted to the search for such aborted fetuses in the paddocks given the diagnostic value of aborted fetuses.

Because the organism is so fragile, it does not survive for long in flushing media. Hence immediate inoculation into transport medium, such as Lander's medium (Lander 1990) is recommended. If this is not feasible, the sample must be at the laboratory within 6 hours. After incubation of the Lander's medium under microaerophilic conditions, it can be subcultured onto blood agar and further incubated under microaerophilic conditions. Differentiating between CFV and CFF is done on the basis of biochemical tests (OIE 2017a).

Serology

Genital campylobacteriosis does not result in a measurable serum antibody response in either males or females. Hence, serological tests based on humoral antibodies are of little or no value.

Serological methods based upon the vaginal IgA response have been widely used as, even though that antibody is not particularly effective at eliminating CFV infection, it is a rapid and persistent response. However, serological methods do not accurately differentiate between CFV and CFF, so in situations in which cattle may have been exposed to CFF (e.g., where cattle and sheep are managed together), the reliability of tests based on vaginal mucous IgA decreases.

Tests based on vaginal IgA include the following:

Vaginal mucous agglutination test: Many authors agree that this test can be very useful at herd level (50% of infected animals will be detected), although results at individual animal level can be very misleading (MacLaren & Agumbah 1988). The test has, however, largely fallen into disuse as it cannot differentiate between CFF and CFV.

Immunofluorescence: Immunofluorescence, alone or in combination with culture, was considered to be a valuable adjunct to the diagnosis of campylobacteriosis. Indeed, Dekeyser (1984) considered it to be 'a quick, convenient and accurate method of diagnosing carrier bulls'. In the past, immunofluorescence was widely used in the diagnosis of campylobacteriosis, but, more recently, its inability to differentiate between CFF and CFV has led to a decline in its use.

IgA ELISA: An indirect ELISA test for the identification of immunoglobulin A (IgA ELISA) in vaginal mucous samples was developed in Australia (Hum et al. 1991). Field evaluation of the test suggested a specificity of 98.5% for the identification of CFV-infected herds (Hum et al. 1994). Where CFF infection of cattle may occur, the cross reactivity between CFF and CFV limits the usefulness of the test (McFadden et al. 2004, Benquet et al. 2005).

Monoclonal antibody ELISA: The limitations of polyclonal antibody ELISAs have led to the development of a monoclonal antibody assay (Devenish et al. 2005). In this assay, samples are exposed to a series of monoclonal antibodies that recognise either components that are present in all *Campylobacter fetus* strains or which are differentially present in CFF and CFV. The assay sensitivity is quoted as being 100% and its specificity of 99.5% relative to the results of culture. This method has been

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adopted by the OIE (2017a) as one of its suggested methods of diagnosing CFV.

Polymerase Chain Reaction

Many of the difficulties of culture and of crossreactivity between CFF and CFV can potentially be circumvented by the use of PCR methods. Over the past decade, PCR tests that have been developed (Hum et al. 1997, Schulze et al. 2006) can differentiate between CFF and CFV in culture. More recently, a sensitive PCR has been developed for use in the field that appears to be able not only to differentiate between CFF and CFV but also to identify the presence of CFV in grossly contaminated samples (i.e., preputial scrapings) or in samples from which culture has not been possible (McMillen et al. 2006). Despite the advances offered by PCR, van der Graaf-van Bloois et al. (2013) were of the opinion that, although PCR methods are able to accurately identify *Campylobacter fetus* to a species level, their specificity and sensitivity was insufficient to accurately separate CFF and CFV.

Prevention, Control, and Eradication

Prevention

Because CFV is only spread through the venereal route (or by contact with contaminated objects), preventative management aims to ensure that infected animals are not introduced into a susceptible herd. Breeding by AI is probably the most effective preventative measure of all, although this is not always feasible, especially in beef herds. For beef herds, preventative measures include (Peter 1997):

- Bulls should not be shared or rented, unless it is absolutely certain that they are disease-free;
- Culled bulls should be replaced with virgin bulls;
- Culled cows should preferably be replaced with virgin heifers, although the risk posed by heavily pregnant or freshly calved cows is small;
- Keeping the breeding season short (2–3 months);
- Avoiding common grazing with animals from other herds and ensuring that fences in and around the farm are stockproof; and
- Where CFV vaccine is available, all incoming bulls should be vaccinated at the time of breeding soundness examination, and all other animals should be vaccinated before the start of the breeding season.

Eradication

Eradication of CFV from infected herds is based on three features of its epidemiology: its transmission is exclusively venereal; bulls remain permanently infected; and that most (but not all) infected cows overcome the infection in a period of 3 to 6 months after the initial infective service.

In situations in which cattle can be bred by AI, this is probably the most effective means of control because incoming uninfected animals do not contract the disease and infected animals eventually become immune and removal of bulls from the herd prevents further venereal transmission of the disease. Although CFV does not survive through a normal gestation in most cows, there are occasional persistently infected animals. It has therefore been recommended that all cows in the herd are bred by AI until every exposed cow has completed two normal pregnancies. Other means of eradication include the following:

Split herd management: Rigid segregation of 'clean' animals (i.e., virgin animals or cattle that have never been mated by an infected bull) and 'infected' animals (i.e., all others), with progressive replacement of the latter by the former, has been recommended, although the effort required to maintain segregation is enormous.

Bull management: Peter (1997) has recommended the culling of older bulls (as these are more likely to be persistently infected), sexual rest, and testing of young bulls before the start of mating.

Antibiotic treatment: Infected bulls can be effectively treated with systemic or topical administrations of dihydrostreptomycin or topical administration of neomycin and erythromycin (Dekeyser 1986). However bulls that have been treated with antibiotics will be susceptible to reinfection if they mate with infected cows. Antibiotics have little beneficial effect in the cow whether administered locally or parenterally.

Vaccination

Vaccination programmes prevent the development of campylobacteriosis in susceptible cows and eliminate infection from affected cows (Schurig et al. 1978, Eaglesome et al. 1986). Vaccination should preferably be carried out 30 to 90 days before breeding commences and, because the immunity is relatively short-term, yearly revaccination before the start of the breeding season should be undertaken (Hoerlein 1980). Dekeyser (1986) reported that vaccinated females conceive normally, although many acquire a vaginal infection if they are served by an infected bull.

Vaccination can be used for prevention and treatment of campylobacteriosis in bulls (Clarke et al. 1974). Some studies (Bispig et al. 1981, Vasques et al. 1983, Hum et al. 1993) have suggested that vaccination alone is not effective at eliminating infection and so have recommended concurrent treatment with topical or systemic antibiotics. Protection of the male can also be achieved by the use of double doses of vaccine given on two occasions (Cortese 1999).

Vaccine failure, when it occurs, may be due to antigenic differences between local and vaccinal strains of CFV (Cobo & Favetto 2014) or to the ability of CFV to evade an IgA response.

Infection With *Campylobacter fetus* subsp. *fetus*

Campylobacter fetus subsp. *fetus* is commonly present in the gastrointestinal tract of cattle and sheep. It is transmitted through contaminated feed and water, and the venereal route is not generally regarded as being a significant means of transmission. It is generally associated with sporadic abortions and is not usually a cause of conception failure. Infection with CFF results in a transient bacteraemia, after which the organism localises in the placenta, sometimes resulting in abortion. The majority of such abortions occur between the fourth and seventh months of gestation (Thompson & Blaser 2000).

The placenta is usually autolysed, indicating that death preceded expulsion by a significant interval. Placental lesions are very similar to, although less severe than, those caused by *Brucella abortus*. Typically, there is necrosis, with yellowish-brown discoloration of the fetal cotyledons and leather-like thickening or oedema of the intercotyledonary allantochorion. Lesions in the bovine fetus are not specific (Kennedy & Miller 1993), a situation that is markedly different from the pathognomonic liver lesions in aborted ovine fetuses (see Chapter 29).

Although the venereal route of transmission is not regarded as of importance for CFF, it has been reported that some strains can be transmitted by that route, resulting in a syndrome that is more like classical venereal campylobacteriosis than the syndrome more

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normally associated with the organism (MacLaren & Agumbah 1988).

Brucellosis

Bovine brucellosis is usually caused by *Brucella abortus. Brucella melitensis,* transmitted from sheep and goats, can also cause disease in cattle. Although *B. suis* has been isolated from cattle in contact with infected pigs, it does not appear to cause disease in cattle (Robinson 2003). *Brucella abortus* affects Bovidae other than *Bos taurus,* including *Bos indicus,* yaks, domestic buffaloes and bison, and wild Bovidae, Cervidae, and Camelidae, with the result that infection can be maintained in wildlife populations. Brucellosis results in abortion, usually in the second half of pregnancy, together with metritis and retained fetal membranes (RFM). In bulls, it can cause orchitis, epididymitis, and infections of the accessory sex glands (Nicoletti 1986).

Brucella abortus occurs in most countries of the world in which cattle are kept in any significant numbers. Because of the enormous losses that the disease causes to dairy and beef cattle industries, it has been the subject of eradication schemes in many countries. Currently, *B. abortus* is found worldwide in cattle-raising regions, except for Japan, Canada, Scandinavia, Luxembourg, the Netherlands, some central European countries, Australia, New Zealand, and Israel, where it has been eradicated (OIE 2017b). The UK is considered to be free of the disease, and most of the states of the mainland US are also free (Yaeger & Holler 2007). The highest incidence is observed in the Middle East, the Mediterranean region, sub-Saharan Africa, China, India, Peru, and Mexico. Currently, countries in central and southwest Asia are seeing a substantial increase in cases (OIE 2017b). The OIE requires that brucellosis is a notifiable disease in each of its member countries.

Brucella infection is a serious zoonosis, as it is readily transmissible to humans, causing an acute febrile illness that may progress to a more chronic form. Human infection is mainly acquired by the oral, respiratory, or conjunctival routes, but ingestion of raw milk products constitutes the main risk to the general public in countries in which the disease is endemic. There is an occupational risk to veterinarians, abattoir workers, and farmers who handle infected animals/carcasses and aborted fetuses or placenta (OIE 2017c).

Aetiology and Pathogenesis

Most herd outbreaks have been caused by the introduction of carrier animals. Infection occurs primarily by ingestion but can also occur via mucous membranes. The principal source of infection is aborting cows in which the fetus, placenta, fetal fluids, and milk are all heavily contaminated. Ingestion of contaminated pasture, bedding, food, or water, or licking an aborted fetus, infected afterbirth, or genital exudate from a recently aborted cow are common means by which transmission occurs. Infection may even occur through the teat by infected milk of another cow or through the vagina by infected semen. Calves can become infected through contaminated milk or at the time of parturition.

The organism colonises the udder and supramammary lymph nodes of non-pregnant animals. In pregnant animals, production of erythritol within the placenta allows rapid multiplication of the bacteria, leading to endometritis, infection of cotyledons, and placentitis. The fetus is aborted 48 to 72 hours after death, by which time a degree of autolysis has occurred. The fetal membranes are very frequently retained. For a day or two before, during, and for about a fortnight after abortion the genital discharge of the infected female is highly infected. When the fetal membranes are retained, the uterus may not free itself of infection until about a month after delivery. After the completion of uterine involution, the organisms colonise the udder and supramammary lymph nodes in which, in the next gestation, infection of the placenta may again occur. The organism may live for months outside the animal body in aborted fetuses or fetal membranes but, when exposed to drying and sunshine, it is soon killed. The infantile uterus becomes infected in a very small proportion of calves that are born alive and survive rearing (Wilesmith 1978).

Clinical Signs

The main sign of brucellosis is abortion, which occurs mainly in the second half of gestation. Earlier abortions may occur at the beginning of an outbreak. Occasionally, fetal death is followed by mummification or maceration rather than abortion. Late aborted fetuses may be born alive but either die shortly after birth or are weak, unthrifty, and at risk of succumbing to calf diarrhoea.

Most cows that abort have RFM. The placenta appears dry, thickened, cracked, and covered by a yellowish exudate in the intercotyledonary areas. Cotyledons appear necrotic and may also be covered with an exudate. RFM is more common in cows that abort in later gestation and those that carry to term. Such animals show delayed involution of the uterus and are prone to secondary bacterial invasion with resultant puerperal metritis.

Diagnosis

Diagnosis is based upon the isolation of *B. abortus* from abortion material, milk, or necropsy material. In addition or as an alternative, specific cell-mediated or serological responses to *Brucella* antigens can be demonstrated (Robinson 2003).

The organism can be identified in stained smears prepared from suspected contaminated material, either using a modified Koster and Ziehl-Neelsen method or a fluorescent antibody technique (Brinley Morgan & MacKinnon 1979). Demonstration of acid-fast organisms provides a presumptive diagnosis, but *Chalmydia* or *Coxiella* can be mistaken for *B. abortus* in stained smears, and the sensitivity is low in milk (OIE 2017c). The organism can be isolated by microbial culture from the fetal stomach of an abortus, from fresh afterbirth, or from uterine exudate.

Diagnosis at the herd level as part of eradication schemes has largely relied upon serological tests upon biological materials such as milk, serum, vaginal mucus, and semen. Serology has also been used to confirm presumptive diagnoses based upon Ziehl-Neelsen staining and/or immunofluorescence. Serological tests that are currently in use for screening or diagnosis include:

- The rose Bengal plate test, which was introduced into the UK in 1970 as the main initial screening test of serum samples in the brucellosis eradication scheme (Brinley Morgan & Richards 1974); and
- The plate agglutination test, which was rated highest, in terms of sensitivity and specificity, of the conventional tests and is better than either rose Bengal or complement fixation tests (Gall & Nielsen 2004).

These two tests are known to have significant false-positive rates (i.e., they identify some non-infected animals as positive), so positive samples are reexamined using a more specific serological method.

- The milk ring test, which detects *Brucella* antibodies in milk, is very useful in screening the presence of brucellosis in herds by collecting bulk milk samples or in individual animals (Robinson 2003).
- Indirect ELISAs can be used for screening or diagnosis. They have the advantage of being cheaper and easier to use than

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many other methods (Gall & Nielsen 2004), and their diagnostic accuracy is at least as good as that of the complement fixation. Some ELISAs allow differentiation between vaccinated and infected animals test (Wright et al. 1993, Nielsen et al. 1996).

- Complement fixation test (CFT) and serum agglutination test (SAT) can be used. The SAT is no longer regarded as suitable for the diagnosis of brucellosis (Robinson 2003), as its specificity and sensitivity are poorer than those of the other tests (Brinley Morgan & MacKinnon 1979). The CFT identifies infected adults sooner after infection the SAT and, as the disease becomes chronic, the titres detected by the SAT tend to fall below diagnostic levels, whereas titres detected by the CFT persist at diagnostically significant levels. The CFT is also more effective than the SAT in differentiating titres arising from infection from vaccination. In calves vaccinated with Strain 19, titres detected by the CFT become negative in most cases by 6 months after vaccination, whereas an 18-month period is required for the SAT.

Molecular methods, including PCR, have also been developed for identification of *Brucella* strains. Differentiation is complicated by a high degree of DNA sequence homology within the genus, but tests are now available which allow identification of different strains (Ocampo-Sosa et al. 2005) and between pathogenical stains and vaccine strains (e.g., S19 or RB51 strains; Garcia-Yoldi et al. 2006, Lopez-Goni et al. 2011). PCR and other molecular methods for the diagnosis of *Brucella* species now appear to be sufficiently rapid, simple, and robust to provide species–specific information and to be useful for the epidemiological study of brucellosis (OIE 2017c).

It should be noted that, during an active infection of a herd, the results of tests should be interpreted with some caution. Negative reactions will occur during the incubation period; furthermore, it is quite common to get a negative reaction at the time of, and for a few days after, a brucellosis abortion. Infected bulls sometimes fail to react to the blood test, and it is considered that, if the agglutination test is performed on seminal plasma rather than blood, a better indication of infection will be obtained.

Control

Eradication

The Food and Agriculture Organization recommends the following sequence of action for eradication of brucellosis from a nation or region (Robinson 2003):

Phase 1: High or unknown prevalence, with no control programme. The first step is to identify the prevalence and distribution of the infection through programmes such as investigation of abortions and surveys of cattle on farm and in markets or at slaughter.

Phase 2: Mass vaccination. In the UK the first mass vaccination was undertaken with vaccines prepared from killed cultures of McEwan's *B. abortus* S45/20. This was later replaced with strain S19, a smooth variant of a strain of *B. abortus* of reduced virulence but of high antigenic quality. Strain RB51 (a rough strain) is also being used for vaccination; its advantage over S19 is that it is less likely to cross-react with serological tests for virulent strains. Vaccination should be supported by checks on and off farm that animals are seropositive.

Phase 3: Test and removal, segregation, or slaughter. Herds are tested for animals that are seropositive to virulent strains; these animals are segregated or slaughtered. Radostits et al. (2007) suggested that the incidence of infection has to be reduced to about 4% of the bovine population before a slaughter-based eradication programme is likely to be feasible. As eradication progresses, the proportion of false positives from the cross-reaction between S19 and virulent strains reaches a point at which it is more cost effective to desist with vaccination. Later in the eradication process, monitoring at a herd level (e.g., using bulk milk) or in markets or at slaughter is more cost effective than individual animal testing. The scheme that was used in the UK during this phase of the process is shown in Fig. 24.4.

Phase 4: Freedom. Criteria that have to be met to allow a region to be officially brucellosis free include that the condition is notifiable, that reactors are slaughtered, that vaccination is not used, and that the national/regional brucellosis infection rate has not exceeded 0.2% for at least 2 years. These criteria are reflected in current European Union regulations.

In the UK, the brucellosis eradication scheme also requires:

- Positive identification of cows and their calves;
- Traceable movements of cattle, so that potential carriers and in-contact animals can be found;
- Secure boundaries to individual farms or to eradication areas in order that uncontrolled movements of animals are prevented;
- Regular testing of all cows, followed by immediate slaughter of reactors, with compensation payment for slaughtered animals to ensure farmers' full participation in the scheme; and
- Isolation and testing of any cows that abort or have premature calvings; in the UK any animal calving at less than 271 days of gestation has to be sampled for brucellosis.

Local Control of an Outbreak

Should an outbreak of brucellosis occur, the disease is controlled on farm as far as possible by strictly isolating any animals that abort. Rigorous cleaning, disinfection, and disposal of infective material are practised. The complete isolation of the reactor from 4 days before calving or abortion to 14 days afterwards is the key to successful reduction in incidence of the disease on the farm. Calfhood vaccination should be performed in these infected herds. When the incidence of infection is sufficiently reduced, the reactors may be slaughtered.

Lastly, in heavily infected herds with current abortion, the spread of infection must be controlled in every possible way. It is best to isolate all parturient or aborting animals from 4 days before to 14 days after parturition. Disposal of infected material, thorough cleansing and disinfection after an abortion, and segregation of reactors are practised. There will be a shortage of young stock on such a farm, and this can be made good by buying in calves from disease-free herds; these calves and all other young stock are vaccinated. When the disease becomes quiescent - as shown by further blood tests - disposal of reactors may begin.

Tuberculosis of the Genitalia

Bovine tuberculosis has been eradicated in many countries of the world. However, before eradication schemes were implemented, it was an important cause of infertility, and thus where bovine tuberculosis still exists, it should always be considered as a possible cause. Infection may reach the tract either by spread from the peritoneum via the uterine tubes, or by penetration of the serosa, or by bloodstream invasion, in which case the endometrium may be involved in the absence of serous or tubal lesions. Occasionally,

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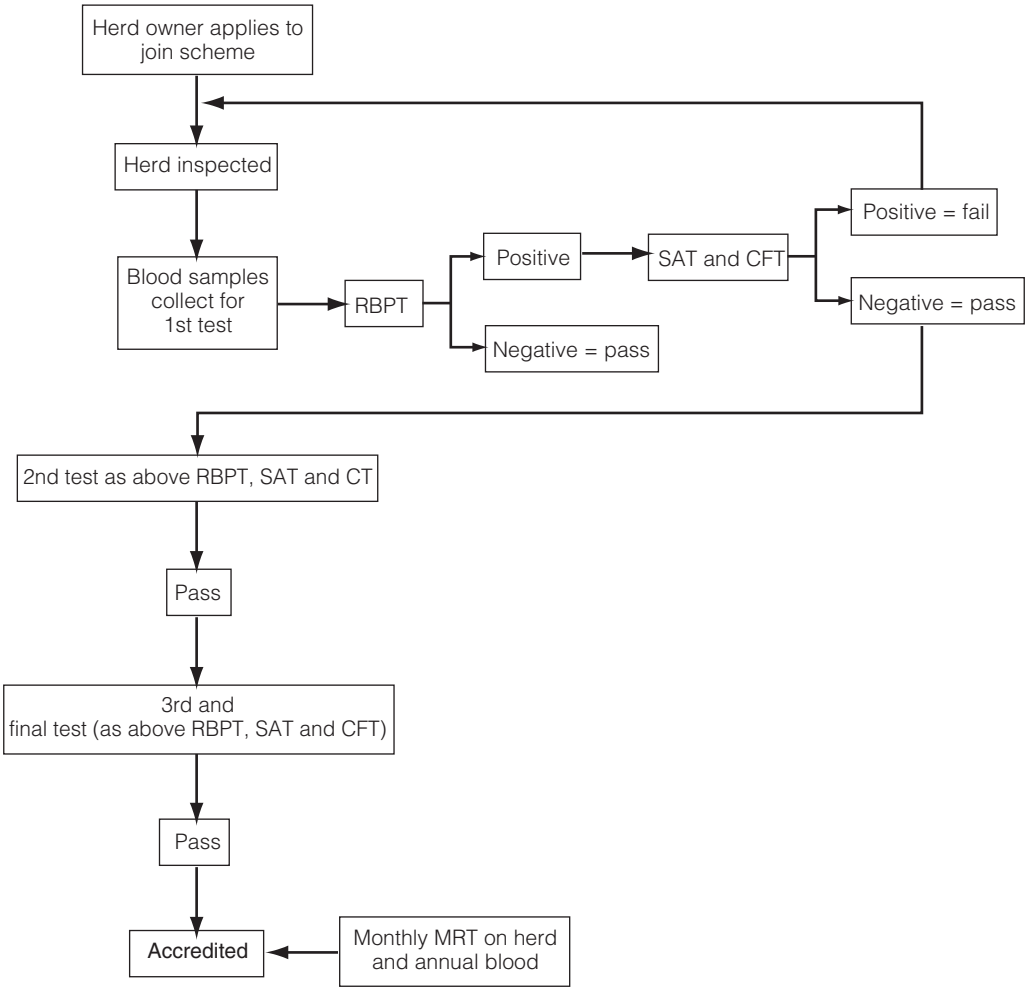

• **Fig. 24.4** Brucellosis testing scheme used in the UK, as part of Phase 3 of the OIE recommendations for the eradication of the disease. (MRT: milk ring test; RBPT: rose Bengal plate test.) (After Brinley Morgan and MacKinnon 1979.)

primary uterine infections may arise from contaminated instruments or hands during gynaecological or obstetrical interferences.

Tuberculosis of the uterus is not an inevitable barrier to reproduction, for it is possible for a calf to be born from a grossly infected uterus (the calf itself being affected by the congenital form of the disease), but it is probable in such cases that the uterine infection was acquired or, at least, developed rapidly, during pregnancy. The disease is also liable to develop in the reproductive organs after parturition.

Leptospirosis

Leptospirosis is an important zoonotic disease of cattle and other mammals that is caused by pathogenical spirochaetes of the genus Leptospira. Leptospires are currently classified into 10 pathogenical species, of which the most important to domestic species are *L. interrogans* and *L. borgpetersenii,* 5 intermediate species, and 6 saprophytic species (Caimi et al. 2017).

Leptospirosis has a worldwide distribution. Large numbers of cattle are affected. In the UK, Ellis et al. (1986) estimated its prevalence at approximately 60% based on microbiology and 27% based on serology. In New South Wales, King (1991) estimated that 27% of cattle were positive to *L. pomona,* 16% positive to *L. hardjo,* and 31% positive to both. In the studies of Hellstrom (1978) and Blackmore (1979) in New Zealand, 81% of herds had active or previous infection with *L. hardjo* and 36% had evidence of *L. pomona* infection.

Each serovar tends to be adapted to a particular mammalian species, known as a maintenance host. Levett (2001) defined a maintenance host as a species in which infection is endemic and in which it is usually transferred from animal to animal by direct contact. In the maintenance host the serovar causes relatively minor symptoms, but more severe disease occurs when infection of an incidental (i.e., non-maintenance) host occurs (Fig. 24.5). The main host-adapted strains of cattle are *L. interrogans* serovar Hardjo type hardjo-prajitno in the UK and *L. borgpetersenii* serovar Hardjo type hardjobovis in the US and Australasia. These serovars will be referred to subsequently in this chapter as *L. hardjo.* Serovars that commonly cause disease in cattle are the pig-adapted strain, *L. interrogans* serovar Pomona (*L. pomona*) and the rodent-adapted strains such as *L. interrogans* serovar Copenhageni. Accidental infection of humans with animal-adapted strains of *Leptospira* results in a severe, flu-like, sometimes fatal, zoonotic disease. Hence leptospirosis is of considerable public heath importance.

Aetiology and Pathogenesis

Infection can enter via skin abrasions or through the mucous membranes of the eye, mouth, or nose. It can also be transmitted

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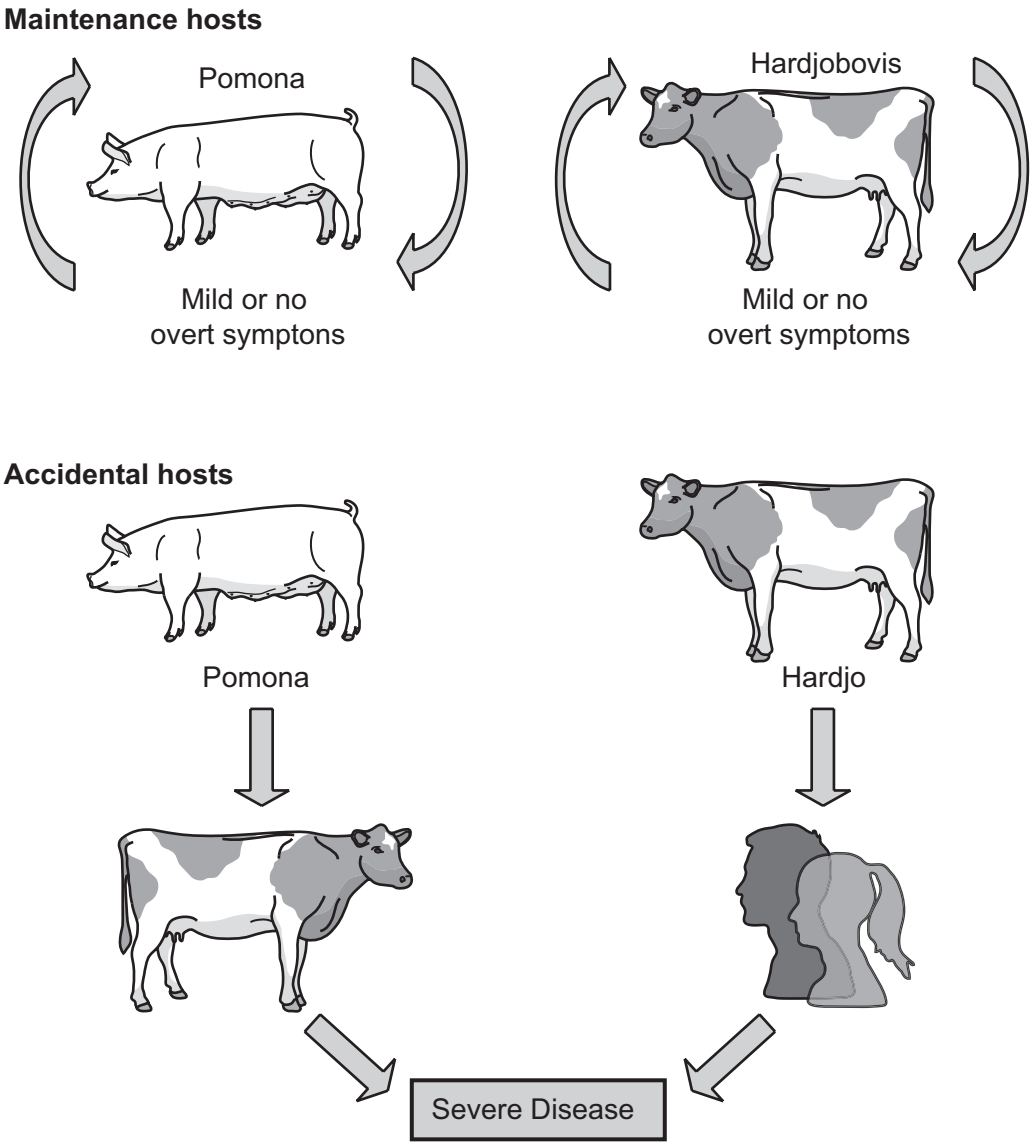

• **Fig. 24.5** Leptospirosis in maintenance and accidental hosts (including humans).

in semen after natural service (Ellis et al. 1986), whereas its presence in vaginal fluids may suggest the possibility of direct venereal transmission (Loureiro et al. 2017). After infection a short latent period (5–14 days) is followed by a bacteraemia that, if caused by a host-adapted serovar (e.g., *L. hardjo*), causes relatively mild symptoms; however, if caused by a non-adapted strain (e.g., *L. pomona*), it results in an acute, severe, sometimes fatal disease. The bacteraemia lasts for some 4 to 5 days, after which the animal mounts an immune response against the leptospires. Thereafter, the organisms localise in tissues that are inaccessible to antibodies, particularly kidney tubules, cotyledons, and fetus (Higgins et al. 1980). Colonisation of the kidney results in excretion of leptospires in the urine for a period of time that lasts between several weeks and the entire lifetime of the animal (Thiermann 1982, Ellis 1984). Renal damage can be severe, which is more serious in non-maintenance hosts than in maintenance hosts. Excretion in the urine provides the major source of environmental contamination and of direct infection both of other cows and of humans. Other pathological changes, such as haemolysis, nephritis, and hepatitis, can also be serious in non-maintenance hosts.

Fetal infection, as a result of placentitis and leptospires crossing the placenta, varies in outcome depending on the stage of gestation. Abortion may result, or the fetus may produce antibodies and survive, or it may be stillborn or be born weak and latently infected. Leptospires can be present in postpartum discharges for up to 8 days (Ellis 1984) and can persist in the pregnant and non-pregnant uterus for up to approximately 150 and approximately 100 days after infection, respectively.

Transmission of leptospirosis depends on conditions favouring the survival of the organism in the environment, on the number of carrier animals in a population, and on the length of time for which carrier animals shed leptospires. The risk of infection increases when there is a high density of carrier and susceptible animals. Survival of leptospires in the environment depends on variations in temperature and humidity. Drying and pH values outside the range of 6–8 are detrimental to their survival in the environment, whereas warm, moist conditions favour its survival. The organism can survive for long periods in wet soil or stagnant water, and the risk of infection with *Leptospira* organisms increases during periods of high rainfall

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or flooding. Effluent is an important source of leptospires for feedlot cattle.

Clinical Presentation

Infection of naïve cattle with *L. hardjo* generally results in a mild disease. Most animals show no clinical signs but become carriers and excrete the organism in the urine. In other animals, clinical signs include the following:

- A transient rise in temperature and/or the appearance of being unwell or inappetent for a few days;
- Mastitis ('flabby bag') or a sudden drop in milk production (Ellis & Michna 1976); and
- Abortion or stillbirth occurring up to 6 or even 12 weeks after the acute phase of an infection. It can occur at any time from the fourth month to term; it is most common after 6 months. Abortion can occur in the absence of any clinical signs of disease (Thiermann 1982).

Infection with non-adapted serovars (notably Pomona, Canicola, Copenhageni, Icterohaemorrhagiae, and Grippotyphosa) results in an acute febrile disease, characterised by temperatures of 40°C or more, together with haemoglobinuria, icterus, and anorexia. Leptospiral mastitis may also be present. Deaths may occur, especially in calves, and there may be sporadic abortions or an abortion storm.

Diagnosis

There are no lesions that are specific for leptospirosis. The placenta of animals that have aborted may exhibit oedema of the intercotyledonary tissue, and the cotyledons may be fawn-coloured and flaccid. Aborted fetuses are usually severely autolysed, although some are quite fresh and oedematous. If fetal interstitial nephritis is present, it is pathognomonic. Antibodies in fetal serum are also diagnostic, but titres are low (~1:10), which requires specialised methods for identification (OIE 2017d).

It is possible to identify *Leptospira* organisms by direct isolation, dark-field microscopy, or fluorescent antibody staining of fetal fluids, kidney, or lung or cow urine. Although definitive, these methods are time consuming and demanding. Diagnosis therefore relies primarily upon serology upon maternal or fetal blood, for which the most widely used test is the microscopical agglutination test (MAT) or, more recently, ELISA. The MAT is the most widely used serological test and is the reference test against which all other serological tests are evaluated. ELISAs have been developed against whole cells and membrane proteins, with moderate success, although validation of the tests remains a constraint to their use (OIE 2017d). Additionally, PCR methods are being developed for the identification of serovars and for the rapid identification of leptospires in tissue/fluid samples. PCR methods are most fully validated for human samples (e.g., Thaipadunpanit et al. 2011) but are being increasingly evaluated for use in field isolates (e.g., Hamond et al. 2014, Martin et al. 2015). Balakrishnan et al. (2015), however, noted that despite recent advances in PCR technology, it remained less accurate than MAT in field samples.

Even using the 'gold standard' MAT, diagnosis is not straightforward. High titres against non-host adapted serovars, together with clinical signs of disease, can be diagnostic, although abortion can occur in the absence of high titres. Adapted serovars are more difficult. Even though active infection with *Leptospira* causes rising titres in paired sera, high MAT titres usually persist only for up to about 3 months after infection. Hence, titres are generally in decline by the time abortion occurs because there is usually an interval of 6 to 12 weeks between infection of the dam and fetal expulsion (Ellis 1984–1985). By that time the antibody titre of the dam is either falling, static, or not detectable. As a result, examination of paired sera (taken at abortion and 2–3 weeks later) from individual animals is of minimal diagnostic value.

TABLE 24.2 Number of cows to be sampled for the diagnosis of leptospirosis

Total herd size	20	40	90	120	300	≥ 450
Number of cows to be sampled	16	21	25	26	28	29

It is therefore preferable to use serology to identify the presence of active infection by using the MAT as screening test in herds in which the disease may be endemic. Numbers of animals from which samples should be collected are suggested in Table 24.2. Interpretation requires differentiation of active infection from vaccine titres:

- Titres of <1:400 probably reflect historical infection or vaccination titres. Most animals have titres <1:100 from approximately 3 months after vaccination.
- Titres of 1:100 are present in 40% to 70% of cattle naturally infected with *L. hardjo* regardless of their vaccination status.
- Titres of >1:1600 are indicative of active infection, but animals can have active infection with titres of <1:100 (Ellis et al. 1982).
- When more than 20% of the herd are seropositive or if titres are >1:1600, active infection is present and further spread of the disease is possible (Anon 1992).
- Titres after acute infection can be >1:25,000, and a significant increase in titres occurs if the samples are collected during an acute episode of leptospirosis.

Treatment and Control

General control measures related to good hygiene, thus minimising the risk of infection with leptospires from other host species, should be implemented. The spread of infection via the environment should also be controlled. There should be strict segregation of cattle from pigs, rodent control, and pig effluent should not be spread on grazing pastures for cattle. Fencing off or draining contaminated water sources are recommended to reduce the environmental load of organisms to which cattle are exposed. Sheep also excrete *L. hardjo* in their urine (Fang et al. 2015). Their role in the epidemiology of the disease in cattle is still not clear because sheep flocks in which cattle and sheep are grazed together show infection with *L. hardjo,* but sheep-only flocks do not (Hashimoto et al. 2010).

There are two methods of specific treatment and control: the use of a vaccine, or parenteral streptomycin/dihydrostreptomycin; or a combination of both. The antibiotic should be used at a dose rate of 25 mg/kg by intramuscular injection. Repeated doses may be necessary. Streptomycin is effective in clearing *L. pomona* from the urine of infected cattle, and treatment with antibiotic plus vaccination has been effective in arresting the progress of an abortion storm. In countries in which streptomycin is not registered for used in food-producing animals, other antibiotics (e.g., tetracyclines, ampicillin, or amoxicillin) can be used. Furthermore, *L. hardjo* may also be less susceptible to streptomycin than to alternative antibiotics (Prescott & Nicholson 1988, Radostits et al. 2007).

In closed herds, vaccination of all members of the herd should be done annually. In open herds, the frequency should be increased to 6-month intervals; this is particularly important for heifers between 6 months and 3 years of age (Ellis 1984). Vaccines are

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based upon bacterins, which produce relatively low antibody titres, but which confer protection for about 12 months. There is little or no crossprotection between the main serovars that affect cattle, so the use of bivalent vaccines (*L. hardjo* and *L. pomona*) or trivalent vaccines (*L. hardjo, L. pomona* and *L. copenhageni*) is common (Radostits et al. 2007). In situations in which the losses due to leptospirosis are low, vaccination may not be cost effective. However, the zoonotic risk of the disease is such that, even when losses are not great, public health authorities may exert considerable pressure to ensure that susceptible cattle are vaccinated. In New Zealand, a very high incidence of human leptospirosis occurred during the 1950s due to the high prevalence of the infection among dairy cows and the high proportion of the New Zealand labour-force who worked in livestock industries (Kirschner & McGuire 1957). The risk of human leptospirosis was considered of such significance that various programmes were introduced to limit the spread of the disease to humans, culminating in a vaccination programme for dairy cows (Oertley 1999). In excess of 90% of New Zealand dairy cows are now vaccinated against serovars Hardjo and Pomona and, when human leptospirosis does occur among farm workers, up to 90% of cases are associated with herds that are unvaccinated (Marshall & Chereshsky 1996).

Salmonellosis

Abortion due to salmonellae has been reported from many countries. Salmonellae may cause abortion as a result of prolonged pyrexia or as a result of infection of the fetoplacental unit.

Aetiology and Pathogenesis

Salmonellae are an important cause of losses in cattle, primarily through enteritis, septicaemia, and abortion. Nomenclature of salmonellae has undergone many changes over the years; most of the 'species' that were once recognised are now considered as serovars of a single species. Thus '*Salmonella typhimurium*' is correctly named '*Salmonella enterica* subsp. *enterica* serovar Typhimurium' (Grimont et al. 2000). In this chapter, salmonellae will be named according to species and serovar, e.g., *Salmonella* Typhimurium.

In Britain, salmonella-induced abortion has persisted as a continuing, although not a major problem, for some time (Table 24.1). The main organism involved is *Salmonella* Dublin, which is responsible for 80% of salmonella abortions (Carrique-Mas et al. 2010). *Salmonella* Dublin is not evenly distributed throughout the world. It is common in the UK (notably Dorset, Somerset, and southwest Wales) and Europe, South Africa, and parts of South America. In the US, it was confined to California and other regions west of the Rockies until recently, but has spread eastwards through the movement of infected cattle (Bulgin 1983, Radostits et al. 2007). Likewise, in Australia, it was confined to the southern states but has now also spread to Queensland (Trueman et al. 1996). *Salmonella* Typhimurium is endemic in cattle throughout the world but is not the major cause of reproductive failure; *S.* Newport is probably the most common of the exotic salmonellae to infect cattle, but a wide variety of other species are isolated during individual outbreaks. In New Zealand, *S.* Dublin is not present, but *S.* Brandenburg is a significant cause of abortion in cattle (Clark et al. 2004).

The disease is contracted after the consuming of feedstuffs or grazing of pasture that has been contaminated with faeces from infected animals, slurry from animal units, human sewage, or infected river water. After infection, there is an initial bacteraemia during which the organism spreads to the liver, spleen, lungs, and lymph nodes of the dam. Infection localises into placentomes 6 to 8 days later, causing a recrudescence of pyrexia. Death of the fetus and subsequent abortion occur as a consequence of the resulting placentitis. The proportion of the herd that is affected depends on the stage of gestation of animals at the time of infection. Commonly, only a small number of animals abort, although significant abortion storms can also occur.

Clinical Signs and Diagnosis

The classical signs of salmonellosis in adult cattle include a marked pyrexia (>40°C), severe diarrhoea, and dysentery, which may be associated with abortion. More frequently, salmonella abortions occur in late pregnancy in the absence of any other clinical signs, although malaise, pyrexia, and inappetence have also been recorded in some animals aborting because of *S.* Dublin infection.

A definite diagnosis depends on the isolation of the organism from fetal tissues and membranes, uterine discharges, or vaginal mucus. Culture of fetal stomach contents and/or fetal brain should routinely be carried out on aborted calves, as this is often the first indication of *S.* Dublin in a herd (Henderson & Mason 2017). Direct culture allows definitive identification of the causal serotype. Faecal culture from dams with clinical signs has only a modest sensitivity (38%), largely because of the very small numbers of organisms that are in the sample.

Serological tests can be used for *S.* Dublin although agglutinins fall to low titres fairly soon after the event (Hinton 1973). However, serology, either of individual animals or of from herd (in bulk milk) have higher sensitivities than faecal culture (80% and 55%–95% respectively; Warnick et al. 2006) and can be used to diagnose the presence of *S.* Dublin in a herd. Carriers are difficult to identify; however, shedding increases during periods of stress, and so there is a better chance of identifying such animals in postcalving cultures. O'Leary (2014) considered that, if the fetus is not available for culture, serology on maternal blood is the next best option for diagnosis.

Control

Cows that have aborted only excrete the organism for a very short period of time, unlike the continuous or intermittent excretion that occurs after enteric infection. Potential excreters need to be isolated until vaginal discharge ceases. Fetuses and fetal membranes, together with contaminated bedding, should be disposed of safely. Adequate cleansing and disinfection of premises should be performed. In the face of an abortion storm, reducing stock density (e.g., by dispersing or set stocking animals around the farm) may help to reduce spread between animals.

Vaccination can be used to control salmonellosis. A killed vaccine that contains *S.* Dublin and *S.* Typhimurium in many countries in which *S.* Dublin is present, and, in New Zealand, a vaccine that contains *S.* Bovismorbificans, *S.* Hindmarsh, *S.* Typhimurium, and *S.* Brandenburg is available. These vaccines can be used to either as a prophylactic measure (in conjunction with other hygiene and biosecurity measures) or to reduce the number of abortions in the face of an outbreak.

Listeriosis

Listeria monocytogenes is primarily a pathogen of the central nervous system in sheep and cattle, in which it causes encephalitis. It is consistently, although not frequently, isolated from bovine abortuses and is also a cause of abortion in sheep and goats (Chapter 29).

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Other species of *Listeria* (*L. ivanovii* and *L. seeligeri*) are also rare causes of abortion in cattle.

Aetiology and Pathogenesis

Listeria monocytogenes is ubiquitous in the environment, being present in the soil, sewage effluent, bedding, and foodstuffs. It persists, as it is particularly resistant to the effects of drying, sunlight, and extreme temperature. The source of infection in cases of abortion is almost always grass silage that is either grossly contaminated with soil, has a low dry matter content, or has undergone inadequate fermentation, resulting in a high-pH, butyric silage. Crossinfection between sheep and cattle is possible, and there is evidence that some individuals become symptomless carriers, excreting the organism in faeces and milk.

The organism gains entry by ingestion or by penetration of mucous membranes of the respiratory system or conjunctiva, as well as the central nervous system. The organism has a predilection for the placenta, causing a placentitis, death of the fetus, and abortion.

Clinical Signs

Abortions are usually sporadic, occurring towards the end of gestation. However, there are rare reports of serious outbreaks and very rare reports of abortion storms in some herds. In some individuals, there may be pyrexia before, at the time of, or after abortions have occurred. It is very uncommon for cows that show neurological signs of listeriosis to abort, and vice versa. The aborted fetus is usually autolysed but generally lacks specific lesions. However, the presence of multiple yellow or grey pinpoint necrotic foci in the liver and cotyledons, similar to those described for sheep, is characteristic of the disease.

Diagnosis

This is dependent on the identification of the organism in the abomasum and liver of the fetus and in the placenta and vaginal discharges, by a direct smear or by immunofluorescence. Culture of the organism is not easy, although a series of subcultures after refrigeration has proved to be successful. Serological tests are not used in its diagnosis.

Treatment and Control

Cows that show clinical signs of malaise and pyrexia may warrant prophylactic treatment with antibiotics to prevent abortion. Prophylactic treatment of the whole herd is contra-indicated due to the sporadic nature of the disease. If the source of poor quality silage can be identified, this should be withheld from cows in the second half of pregnancy.

Histophilus somni

Histophilus somni (formerly *Haemophilus somnus*) is a common inhabitant of the genital tracts of male and female cattle. The organism can be routinely isolated from the mucosal surfaces of the urogenital tract of normal healthy cattle in the absence of any macroscopic lesions (Eaglesome & Garcia 1992). There are reports of the organism being isolated from 28% of normal cows (Slee & Stephens 1985) and 90% of normal bulls (Janzen et al. 1981). *Histophilus somni* also infects sheep, but the strains differ between cattle and sheep so that crossinfection between species does not occur (Ward et al. 1995). *Histophilus somni* causes a number of syndromes in cattle (Radostits et al. 2007):

- Septicaemia
- Polyarthritis
- Pneumonia/pleurisy
- Thrombotic meningoencephalitis
- Reproductive disorders (strains *of H. somni* affecting the reproductive tract differ from those that cause systemic disease) (Szalay et al. 1994)
- Endometritis
- Vulvovaginitis and cervicitis (Patterson et al. 1984, Stephens et al. 1986)
- Granular vulvovaginitis (a differential diagnosis of ureaplasmosis) (Roberts 1986)
- Infertility associated with early embryonic death (Kaneene et al. 1987, Ruegg et al. 1988) and abortion (Stuart et al. 1990)
- Testicular degeneration, orchitis, epididymitis in the bull (Corbel et al. 1986, Jubb et al. 1993, Barber et al. 1994)

Histophilus somni is a relatively uncommon cause of abortion in cattle, having been recorded in 0.4% of diagnosed abortions in New Zealand (Thornton 1992) and 1.7% to 3% of abortions in Germany (Kiupel & Prehn 1986). Lesions of the aborted fetus and placenta are non-specific: typically an acute, non-suppurative placentitis mainly within the cotyledons (Jubb et al. 1993). Diagnosis can be made by culture of the organism, which can be difficult because of overgrowth by contaminants. Recognition of the organism may not always be straightforward, as it is pleomorphic. In addition, *H. somni* may be present alongside other potential abortive organisms, making it difficult to be certain whether it was the causal organism (Headley et al. 2015). Serological tests are currently unreliable.

There are few reports on the treatment of infected cows. Penicillin and streptomycin have been reported to be successful in treating cows in which *H. somni* was frequently isolated from cervicovaginal mucus and in which fertility was depressed (Eaglesome & Garcia 1992). Because the organism colonises the genital tract of the bull and can be isolated from semen, this may well be a source of infection of cows and heifers. Good hygiene and the use of combinations of antibiotics should control infection after AI. There is a recent report of *H. somni* being transmitted via embryo transfer and causing the death of transferred embryos (Scarcelli et al. 2004).

Bacillus licheniformis

Abortion due to *Bacillus licheniformis,* which was first identified in the UK in northern Scotland, Cumbria, and Northern Ireland (Counter 1984–1985), has been reported from many countries of the world. Although B. *licheniformis* is ubiquitous, the main source of infection is silage, or water, other foodstuffs, and bedding that become contaminated with silage effluent. Wet, spoilt hay can also be a source. The method of infection is not known, but it is probably haematogenous after entry via the gastrointestinal tract.

Sporadic cases occur in the third trimester of gestation and most commonly in the winter months (Agerholm et al. 1995). There are also reports of small outbreaks in two consecutive years (Counter 1984–1985). Sometimes live calves can be born with some evidence of placental lesions. *Bacillus licheniformis* causes a necrosuppurative placentitis, in which the allantochorion is dry, leathery, and yellow or yellowish-brown in colour, is sometimes oedematous, and has necrotic foci 2 to 3 mm in diameter. When the fetus has been infected, there will usually be evidence of a bronchopneumonia, fibrinous pleurisy, pericarditis, and peritonitis. Purulent exudate is often present in the airways. There are no systemic signs of disease in the cow (Counter 1984–1985). Diagnosis is by culture from

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the fetus (especially the abomasum), placenta, and vaginal swab. Isolation of *B. licheniformis* in the absence of tissue lesions in the fetus may be the result of sample contamination.

The only means of control is to avoid feeding infected silage or hay.

Other Bacterial Causes of Infertility

Many other species of bacteria (notably *Trueperella pyogenes, Aeromonas* spp., *Fusobacterium necrophorum, Escherichia coli,* and *Streptococcus* spp.; Table 24.1) are periodically isolated from bovine fetopathies (Rowe & Smithies 1978, Moorthy 1985, Smith 1990). These organisms are not regarded as primary pathogens but may be accidental contaminants of the uterus, probably after haematogenous spread in the dam.

Abortions may occur at any stage of gestation, although most commonly in the last trimester. Diagnosis is usually made by the isolation of the organism from the placenta, abomasal contents, or fetal tissues from which the organism is isolated in pure growth of bacteria from fetal viscera (particularly liver and lung) or stomach contents. It is very probably that it was the cause of the abortion, especially if lesions consistent with a bacterial infection are present and other causes for abortion have been ruled out. Because the abortions are sporadic, there are no suitable methods of treatment or control.

Mycoplasma, Ureaplasma, Acholeplasma, and *Chlamydia* Infections

There are many species of *Mycoplasma, Ureaplasma, Acholeplasma,* and *Chlamydia,* which are found as commensals of many species of animals. They have been implicated in disease in as much as they are commonly isolated from diseased tissue. However, they are also found in healthy tissues, and experimental studies to demonstrate pathogenicity are relatively limited (Eaglesome & Garcia 1992), so it is more likely that in most situations they are opportunistic, rather than primary, pathogens. For example, Ball et al. (1978) recovered mycoplasmas from 23.7% of aborted placental material and none from normal controls, and from 4.4% of aborted fetuses and 1.3% from non-aborted controls. On the other hand, infections with various *Mycoplasma* species and with *Ureaplasma diversum* have been associated with female infertility, abortion, and male infertility, and for some species (notably *M. bovigenitalium* and *M. bovis*) there is conclusive evidence of a role as a pathogen.

Spread of mycoplasmas and ureaplasmas is largely through infected semen or via the respiratory route. It is therefore better to use AI rather than natural service until bulls are known to be free of infection. However, because AI may also play a role in the spread of infection, it is suggested that the standard Cassou pipette should be protected by a disposable polythene sheath to prevent vulval or vaginal contamination before it is introduced through the cervix. The uterus can be infused with a solution containing 1 g of tetracycline or spectinomycin (where these are licenced for use in cattle) one day after insemination, a treatment that has been shown to improve pregnancy rates. A number of antibiotics have been incorporated in semen for the control of these organisms. A combination of lincomycin, spectinomycin, tylosin, and gentamicin added to raw semen, and non-glycerolised whole-milk- or egg-yolk-based extenders have been shown to control *M. bovis, M. bovigenitalium,* and *Ureaplasma* spp. (Shin et al. 1988).

Mycoplasma bovigenitalium

Mycoplasma bovigenitalium is commonly found in the vaginal mucus of normal cows (Trichard & Jacobsz 1985), repeat breeders, and subfertile cows in which no other cause of infertility could be determined (Langford 1975, Nakamura et al. 1977, Kirkbride 1987). The organism may also cause granular vulvovaginitis (Afshar et al. 1966, Irons et al. 2004), although the evidence for its role in natural occurrences of the disease is not unequivocal, and it has been suggested that a considerable degree of strain-to-strain variability in pathogenicity exists (Saed & Al-Aubaidi 1983).

Mycoplasma bovigenitalium is also a common isolate from semen and preputial washes of bulls (Fish et al. 1985, Kirkbride 1987), and spread of the organism from affected bulls to cows has been demonstrated. It has been implicated as a cause of seminal vesiculitis, as it is both isolated frequently from clinical cases and can infect the vesicular glands after experimental inoculation. When it infects the testes or epididymides, it may cause detrimental changes to semen quality, especially after cryopreservation.

Mycoplasma bovis

Mycoplasma bovis is widespread, but not ubiquitous in cattle populations; it is commonly isolated from cattle in the UK and US and is present in Australasia (Nicholas & Ayling 2003, Ayling et al. 2004). It causes respiratory disease, otitis media, and polyarthritis in calves (Henderson & Ball 1999), mastitis in adults (Kirk et al. 1997), and infects the female genital tract (Irons et al. 2004). It is a successful colonist of the vagina and uterus, causing persistent infections (for 1 and 8 months respectively), extensive endometritis, salpingitis, and even peritonitis. Infection occurs through the respiratory, mammary, or male and female genital tracts. It can be spread through AI with infected semen: Stipkovits et al. (1983) recorded seroconversion of cows that had been inseminated with contaminated semen that subsequently aborted. Infection of the male can cause orchitis and seminal vesiculitis and affects semen quality (Nicholas & Ayling 2003). Natural and experimental infections cause abortions (Stalheim et al. 1974, Bocklisch et al. 1986) and the birth of premature calves.

Diagnosis of *M. bovis* can be made by serology, PCR, or culture from individual animals, or by bulk milk serology or PCR for the identification of infected herds. ELISA can be used for serology on individual animals but is better used to identify seroconversion or high titres in groups of animals. It can also be used in bulk milk. Alternatively, the organism can be demonstrated in bulk milk or pathological specimens using PCR. Culture is the definitive means of identification, but is technically demanding and has a risk of providing false negative results. Samples for culture require careful handling, including transport in specialised media (Maunsell et al. 2011). Lesions in abortions can include mild through to severe placentitis, although not every case from which *M. bovis* is isolated presents with placental lesions. Likewise, fetal lesions can range from placentitis, suppurative fetal bronchopneumonia, fibrinous peritonitis, myocarditis, and epicarditis through to no gross lesions (Yaeger & Holler 2007, Watson et al. 2012). Although it is seldom found in the reproductive tract of normal cows, isolation of the organism from the placenta or aborted fetus can be considered significant (Kirkbride 1990).

Control of *M. bovis* abortion is difficult as (i) it is sporadic, (ii) there is a very limited range of antimicrobial agents that are both efficacious against mycoplasmas and are licenced for use in food-producing animals, and (iii) there is a dearth of vaccines that have demonstrated efficacy against *M. bovis* abortion. Maintenance of a closed herd is considered the best way of preventing its infection

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with *M. bovis* (Gonzalez et al. 1992), although its potential for transmission through AI semen can nullify even this method.

Other *Mycoplasma* Spp.

Other *Mycoplasma* species (e.g., *M. alkalescens, M. arginini, M. bovirhinis, M. californicum, M. canadense, M. mycoides* subsp. *mycoides* (LC), and Group 7 *Mycoplasma*) have been isolated from abortuses, the genital tracts of cows and bulls, and semen (Boughton et al. 1983, Kapoor et al. 1989, Gilbert & Oettle 1990, Hum et al. 2000), usually with no evidence of consequential pathology. Hassan and Dokhan (2004) therefore suggested that interpretation of a causal role for these organisms in an abortion should rest upon demonstrating the presence of lesions in the fetus that are compatible with the known pathological effects of mycoplasmas.

Mycoplasmas are fragile organisms that require careful handling for successful microbiological culture. Specialised transport media are therefore recommended (Yaeger & Holler 2007). Provided samples are appropriately collected; most bovine mycoplasmas can be recovered using conventional *Mycoplasma* media, although some may require special supplements or conditions for optimum growth (Eaglesome & Garcia 1992).

Ureaplasma diversum

Ureaplasma diversum is a common inhabitant of the genital tract of the cow. It is most commonly found in the vagina and vestibule, but it persists only briefly in the uterus and uterine tubes. It also affects male animals, resulting in seminal vesiculitis, granular balanoposthitis, and impaired semen morphology (Marques et al. 2009). In cows *Ureaplasma diversum* infection has been associated with granular vulvovaginitis (Schweighardt et al. 1985, Rae et al. 1993, Farstad et al. 1996). Acute infection produces granules around the clitoral region and on the lateral walls of the vagina, which are accompanied by hyperaemia of the vulva and a profuse, mucopurulent vaginal discharge. Large, purulent lesions may also be present, which resemble those of infectious pustular vulvovaginitis (IPV, bovine herpesvirus–1, BoHV-1: see later in this chapter). These may give way to less obviously inflamed, chronic lesions. Gaeti et al. (2014) identified *U. diversum* in cattle with pustular vulvovaginitis that were negative for BoHV-1, although other cattle that had no lesions also had the organism within the vestibule.

Samples should be collected from areas of active inflammation for diagnosis of granulomatous vaginitis. For the diagnosis of abortion, samples should include lung, placentome, stomach contents, and amniotic fluid (Yaeger & Holler 2007). If *Ureaplasma* has caused the abortion, the placenta is likely to be thickened and opaque, accompanied by a mononuclear inflammatory infiltrate, fibrosis, and interstitial necrosis with the lungs displaying a non-suppurative alveolitis with mononuclear inflammation of the tissues surrounding the airways (Jubb et al. 1993).

Ureaplasma diversum can also produce endometritis and salpingitis (Kirkbride 1987). These lesions have been associated with high levels of embryonic death and returns to oestrus, which are accompanied by a mucopurulent vaginal discharge. Abortions may also occur as may the birth of weak calves. Abortions usually occur in the last trimester of pregnancy. Macroscopic lesions of the placenta include thickening of large areas of the amnion and intercotyledonary zones of the allantochorion. The thickened regions are opaque white/yellow, with foci of fibrin exudation and haemorrhage. Usually, no gross lesions are seen in the fetus, but lesions can occur in fetal conjunctivae and lung. These lesions, together with microscopical lesions in the placenta, are quite characteristic, but not pathognomonic, for *U. diversum* abortions. On the other hand, *Ureaplasma* may often be isolated as an incidental finding from calves that have been aborted for other reasons, although differences in virulence of strains probably account for the presence of the organisms in normal reproductive tracts. Hence unless there are histological lesions in the abortus that are characteristic of ureaplasmosis (Murray 1992) or the presence of a virulent strain is demonstrated, *Ureaplasma* isolations should be interpreted with a degree of caution. *Ureaplasma diversum* can be isolated by culture, although it is a difficult organism to grow. It can also be demonstrated by PCR, which may also be of value in discriminating between the more and less virulent strains (Marques et al. 2011). Multiplex PCR may also be of value in isolating *U. diversum* from samples containing mixed infective agents (Tramuta et al. 2011).

The main means of transmission of the infection is by the venereal route. Infected semen used in AI seems to be of particular importance, since its deposition into the uterus allows the development of chronic endometritis rather than of acute vulvovaginitis. However, infection of virgin females and males has been described and it has been suggested that direct transmission between females, or even transmission by dogs sniffing the vulvas of cows (Doig et al. 1979), may occur. Whether it is transmitted between bulls is uncertain.

Acholeplasma spp.

Three species of Acholeplasma have been isolated from cattle: *A. modicum, A. laidlawii,* and *A. axanthum* (Kirkbride 1987). Of these, *A. laidlawii* has been isolated most often, largely from the bull. The current consensus is that *Acholeplasma* is a non-pathogenical commensal.

Chlamydia

Chlamydiae are Gram negative, obligate intracellular parasites. Mammalian strains of *Chlamydia psittaci* have long been associated with abortion and infertility, but until recently, the literature has been fragmentary and complicated. Recent reclassification of the Chlamydiaceae into species that are more closely aligned with host species and disease syndromes has increased the complexity of nomenclature in the literature, but has also clarified understanding of their role in bovine infertility. There has also been confusion with the condition of foothill abortion (see Chapter 28) because at one time, this was believed to be caused by *C. psittaci.* Because both abortion due to *C. abortus* and foothill abortion are sometimes called epizootic bovine abortion, there remains a degree of confusion in the literature.

Chlamydiae were classified into two genera, *Chlamydia* and *Chlamydophila,* in 1999. After some years, these genera were reunited into the genus *Chalmydia* (Pillonel et al. 2015), partly as a result of disagreements over the 1999 classification (e.g., Stephens et al. 2009). In consequence, there is much confusion in the literature over the nomenclature of the chalmydiae that affect domestic animals. In this section the convention will be adopted to refer to all species by their current genus title, *Chlamydia,* regardless of the nomenclature used in the original papers. The genus contains three species that are commonly associated with reproductive disorders in cattle – *C. pecorum, C. abortus,* and *C. psittaci* – and other species (e.g., *C. pneumoniae, C. gallinacea, C. suis*) that are commonly found in cattle (Li et al. 2016) but are not characteristically associated

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with reproductive disease. The order Chlamydiales also contains the recently discovered organism *Waddlia chondrophila* (Livingston & Longbottom 2006), which may also affect reproduction in cattle.

Chalmydiae are probably ubiquitous in cattle (Reinhold et al. 2011), although the role of chlamydial agents in bovine fertility has not been considered as a major threat to cattle industries (Livingston & Longbottom 2006). Many animals appear to be carriers of the infection (DeGraves et al. 2003), but the incidence of abortion due to the organisms appears to be low. Development of more sensitive diagnostic tests may lead to greater significance being attributed to *Chlamydia* (Borel et al. 2006) but the question of the presence of the organism versus its role as a pathogen is often obscure.

Chlamydiae are shed in many secretions and excretions, including vaginal, ocular, and nasal fluids, semen, and urine and so forth, with faecal shedding being the most important route. Organisms are also present in aborted material, including the fetus and placenta (Kaufold et al. 2014a). Infection usually occurs through inhalation or ingestion, including from abortus material, and venereal infection may possibly occur.

Control of chlamydial infections is difficult. In theory, tetracyclines or macrolides could be used to treat pregnant cows that have been exposed to infection, but this is not really practicable because it requires knowing that the secondary chlamydaemia has not occurred, and animals must be treated until normal calving. Moreover, it is likely that antibiotic treatment may promote persistent/latent infection (see Reinhold et al. 2011), which would mitigate the use of such treatments. An increased risk of chlamydial disease is associated with poor herd management and hygiene, but causal links have not been established (Reinhold et al. 2008). Pregnant animals should be segregated from potential sources of infection, particularly abortus material. Vaccines are available for use in sheep but none has yet been developed for use in cattle.

Chlamydia abortus

Chlamydia abortus occurs in cattle, sheep, and other domestic ruminants throughout most of the world, particularly Europe, the US, and the Indian subcontinent. In sheep the organism is responsible for ovine enzootic abortion (see Chapter 29). Infection is probably by ingestion of infected material, but venereal spread via the semen of infected bulls also seems probable (Storz et al. 1976). After infection of the cow, there is colonisation and replication in the endometrium, resulting in endometritis (Bowen et al. 1978) and embryonic death (Yaeger & Holler 2007). The incubation period for the disease is variable, ranging from 5 to 125 days in experimental infections (Storz & McKercher 1962). Moreover, some animals become infected and abort in the same season, whereas others become infected in one season, remain infected, and abort in the subsequent season (Aitken 1983). Reinfection of cattle previously exposed to the organism also results in reduced fertility. On the other hand, carrier states appear to be common, even in virgin animals (DeGraves et al. 2003, 2004).

Abortions usually occur after the seventh month of gestation but have been reported from the fifth month. Abortions are more likely to be sporadic in cattle than in sheep, although abortion storms of up to 20% of pregnancies have been reported. Infection in the last trimester of pregnancy may also result in the birth of live, weak calves. Most cows show no signs before abortion, but experimental infection has resulted in an intermittent, mucoid, vulvar discharge, together with transient diarrhoea, pyrexia, and lymphopenia. Retention of the fetal membranes after abortion is common. Infertility that is not associated with abortion can also occur: Kaltenboeck et al. (2005) demonstrated that subclinical, non-venereally transmitted infection with *C. abortus* has an influence on fertility and suggests that infertility can be caused by reinfection with the organism.

In bulls, *C. abortus* infection can cause epididymitis, seminal vesiculitis, and testicular degeneration (Storz et al. 1968), which may progress to atrophy. It can also be found in clinically normal bulls (Teankum et al. 2007).

Humans who work with aborting ewes can become infected with *C. abortus,* and abortions have occurred in pregnant women (Aitken & Longbottom 2004). Presumably the same could also occur by contact with aborting cattle.

Chlamydia pecorum

Chlamydia pecorum is an endemic intestinal species in cattle (Li et al. 2016), which can also cause systemic illness or affect fertility. Infection can result in severe diseases, including polyarthritis, enteritis, keratoconjunctivitis, pneumonia, and sporadic bovine encephalomyelitis (Livingston & Longbottom 2006). Infection is also associated with uterine infection, infertility, and abortion. Infection may result in endometritis, although more severe disease, including metritis and salpingitis, has resulted from experimental infection (Wittenbrink et al. 1993a, b, Jones et al. 1998). Pyrexia, malaise, and/or a purulent vaginal discharge (Livingston & Longbottom 2006) and low grade/subclinical vaginitis (DeGraves et al. 2003) have also been reported.

Waddlia chondrophila

Waddlia chondrophila is a member of the order Chlamydiales, which has been implicated in bovine abortions. It has been isolated from a number of aborted fetuses (Barkallah et al. 2014), and serological responses to the organism has been associated with pregnancy failure (Dilbeck-Robertson et al. 2003). Moreover, *W. chondrophila* has been identified in the placentas of miscarried human pregnancies (Wheelhouse et al. 2016). Crawford et al. (1989) described the effects of *W. chondrophila* on a bovine fetus after direct administration of the pathogen caused death of the fetus within 2 weeks. Wheelhouse et al. (2016) also administered the organism to pregnant heifers: they failed to cause abortion, but placentitis was identified in one heifer and the organism was isolated from the affected placenta. It is therefore feasible, but unproven, that *W. chondrophila* could be a source of sporadic abortions in cattle.

Diagnosis

The gross pathology of abortions due to chlamydiae in cattle is similar to that occurring in ovine enzootic abortion. The intercotyledonary areas of the placenta are more frequently affected, being thickened and leathery in appearance with a reddish-white, opaque discoloration oedema is quite common. In the aborted fetus, the liver is enlarged with a coarsely nodular surface, a firm consistency and a mottled reddish-yellow colour (Shewen 1986). The fetus may have a pot-bellied appearance due to ascites, and there may be subcutaneous oedema.

The organisms can be cultured from aborted fetuses and discharges after the use of transport media. Tissue can be used to prepare Giemsa-stained smears for the identification of elementary bodies or inclusions. Most infections are now diagnosed by antigen detection ELISA or immunostaining (Aitken & Longbottom 2004) or by PCR (Borel et al. 2006, Menard et al. 2006), which can be used for identification of chlamydiae in the field or after culture.

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Serological tests include the complement fixation test and ELISA, but such methods lack specificity.

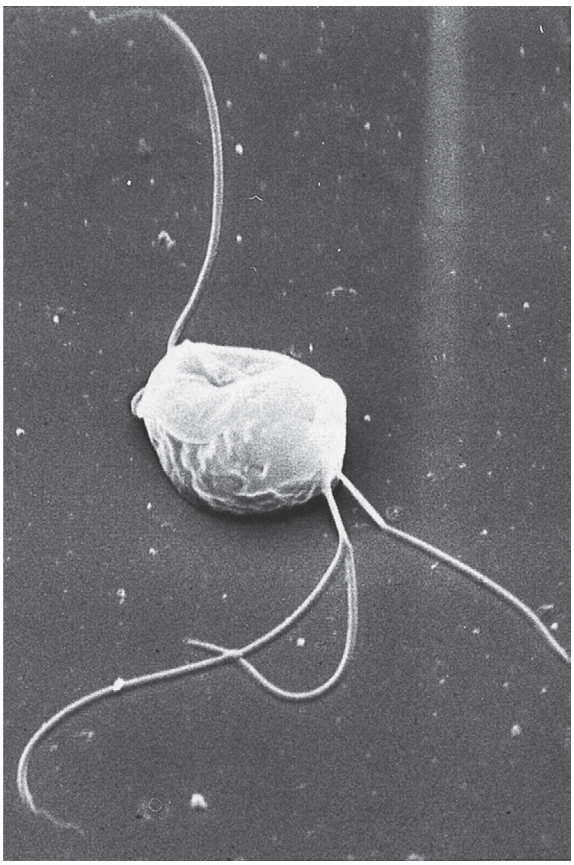
Protozoal Agents

Trichomonosis

The recognition of *Tritrichomonas* (formerly *Trichomonas) foetus* infection as a cause of infertility was an important advance in our understanding of the role of specific venereal pathogens in cattle (Riedmuller 1928). Enzootic trichomonosis was brought under control in the dairy herds of many countries by the widespread introduction of AI during the 1950s and 1960s. However, worldwide, trichomonosis remains a major cause of reproductive failure that is present at a high prevalence in geographical regions in which natural service is the predominant means of cattle breeding. Characteristically, this more commonly involves beef than dairy herds, but the prevalence of infection can be high where dairy herds are bred by natural service. A high prevalence has been reported from many countries, including some states (e.g., California and Florida; Skirrow & BonDurant 1988, BonDurant et al. 1990, Rae et al. 2004), but not all (Grotelueschen et al. 1994, Fox et al. 1995) states of the US, Canada (Copeland et al. 1994), South Africa (Eaglesome & Garcia 1992), Brazil (Jesus et al. 2003), and Australia (Dennett et al. 1974). Geographical isolation has permitted the virtual eradication of trichomonosis in the UK and New Zealand, yet even in these countries, occasional reemergence of the disease can occur from time to time (Taylor et al. 1994, Oosthuizen 1999). Hence whenever natural service is used, trichomonosis must not be overlooked as a cause of infertility.

Aetiology and Pathogenesis

Tritrichomonas foetus is an obligate venereal pathogen. The parasite can be identified by the presence of three anterior flagellae and a characteristic undulating membrane that is visible because of its wave-like motion on one side of the organism when viewed under phase contrast or dark field microscopy (Fig. 24.6). In wet preparations its characteristic jerky, rolling motion can be seen at 100x or 250x magnification.



• **Fig. 24.6** Scanning electron micrograph of *Tritrichomonas foetus* (16,500x).

TABLE 24.3 Relationship between age and *Tritrichomonas foetus* infection in Californian beef bulls

Age of Bulls (years)	Number of Bulls	No. Infected	% Infected
<2	38	0	0
2	221	1	<0.5
2	137	7	5.1
4	156	5	3.2
5	86	8	9.3
6	55	7	12.7
>6	31	2	6.5
SUMMARY			
<2	259	1	<0.4
>2	465	29	6.2

From: BonDurant et al. (1990).

The Bull

Tritrichomonas foetus causes an asymptomatic infection of the bull, residing in the crypts of the penile integument and preputial mucosa. Once infected, bulls can remain lifelong carriers. Younger bulls are less liable to become persistent carriers than older bulls because the crypts of the penile integument and preputial mucosa are less developed in younger than older animals (Peter 1997). Hence, bulls that are less than 3 years old are likely to have short-term infections or to be transient carriers, whereas older animals are more likely to become persistently infected and to perpetuate the infection from season to season (Table 24.3). Infection of bulls usually occurs as a result of coitus with an infected cow; however, it can also be transmitted though infected semen collection equipment. It has been suggested that bull-to-bull transmission could occur through bulls riding each other, but there is no conclusive evidence for the spread of infection in this way.

The Cow

Persistently infected bulls are the primary source of infection, although there can be passive transfer from a previously uninfected bull that has recently served an infected cow (Rae & Crews 2006). Infection can also be disseminated by AI with infected semen, as *T. foetus* survives routine processing of semen for AI. Very rarely, cows can be infected via fomites, such as a contaminated vaginal speculum. Although the number of trichomonads needed to establish an infection in the cow is large (probably several thousand; Clarke et al. 1974), transmission rates are high. Under conditions of heavy work, the number of trichomonads present in the preputial area of

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the bull is reduced, so transmission may be less than 100%; but under normal conditions, it is common for virtually every cow that is mated by an infected bull to become infected.

Tritrichomonas foetus colonises the uterus, cervix, and vagina but survives poorly on the vulva. It causes a mild catarrhal endometritis and vaginitis, with oedema of vulva, perivaginal tissue, and uterine wall. It does not generally invade through the epithelial surface. The disease does not prevent fertilisation but causes embryonic death, resulting in an irregularly extended return to oestrus. Many pregnancies fail at between 30 and 50 days of gestation (Parsonson et al. 1976), although some may occur at a sufficiently advanced stage of gestation to be recognised as an early abortion. It has been suggested (BonDurant 1997) that embryonic death occurs as a result of damage to the developing placentomes. A few animals exhibit normal or even short returns to oestrus. Embryonic death is not infrequently (up to 10% of cases) accompanied by the development of pyometra, in which the uterus is filled with enormous quantities of trichomonad-filled, thinnish pus. Vaginal discharge of this pus is common.

Antibody-mediated (IgG and IgA) immunity develops over several months (Skirrow & BonDurant 1990), so after a series of returns to oestrus, cows develop sufficient immunity to maintain a pregnancy to term. Many cows experience a series of embryonic deaths before they become pregnant and carry the calf to term. Epizootics of the disease in the 1940s were characterised by an average of five returns to oestrus before conception occurred (Bartlett 1948). The time taken for clearance of infection from affected cows is very variable, ranging from 95 days in heifers to 22 months (Parsonson et al. 1976, Skirrow & Bon Durant 1990). Most cows that calve have successfully eliminated the infection and do not pose a risk as long-term carriers, but some animals become persistently infected (i.e., through a gestation and into the next breeding season; Rae & Crews 2006) and act as a reservoir of infection to the herd (Skirrow 1987). Immunity is short term, probably lasting a maximum of 15 months (Clarke et al. 1983a), so that cows are fully susceptible to infection in successive seasons.

Clinical Signs

Introduction of trichomonosis into a herd is associated with a disastrous decline in fertility, with extended returns to oestrus, long inter-calving intervals, and an increased proportion of animals culled for failing to conceive. The presence of pyometra and early gestation abortions is also suggestive of the presence of the disease. Animals that fail to conceive often exhibit mucopurulent vaginal discharge at the time of return to oestrus, and manipulation of the uterus often provokes a discharge from the vulva in which motile trichomonads can be demonstrated. Likewise, the uterine contents in pyometra after *T. foetus* infection are usually voluminous, fluid, odourless, and greyish-white and contain trichomonads in great numbers. Infected cattle therefore have the following clinical presentations:

- Become pregnant and carry to term without clinical signs of infection developing;
- Return to multiple services but show no obvious signs of infection and oestrous cycles may be regular or irregular;
- Fail to become pregnant and develop an oedematous condition of the endometrium with a mucoflocculent discharge;
- Become pregnant but abort at 2 to 4 months of gestation; and
- Develop pyometra and become acyclic.

Some abortions occur between the second and fourth months of gestation, but very few occur after the fourth month. In later term abortions, trichomonads can be found in the chorion, fetal lung, and fetal gut. The fetus is smaller than is appropriate to the period of gestation because of growth retardation. In such abortion cases, the fetus, which is grey in colour, is generally expelled complete in its membranes. There are no signs of putrefaction and *T. foetus* can readily be demonstrated in fetal fluids. Parasites quickly disappear from the vaginal discharges after abortion (usually within 7 days).

Diagnosis

Diagnosis of trichomoniasis is much easier than that of campylobacteriosis. A positive diagnosis of trichomoniasis depends on a demonstration of live *T. foetus* organisms from specimens obtained from the genital tract of female cattle, preputial material of bulls, or aborted fetal and placental tissues.

Diagnosis in the cow is best achieved by demonstrating the presence of trichomonads in uterine pus, vaginal discharges, cervical mucus, or abortus material. The best source of material is the fetal membranes or the organs of an aborted fetus (especially the abomasum). Elimination rates of infection are highly variable after an infected mating, so failure to demonstrate the presence of the organism does not necessarily imply its earlier absence. Material contaminated with faeces should be discarded because non-pathogenic trichomonad-like organisms (Taylor et al. 1994) may be present. In the bull, diagnosis is made by the collection of preputial scrapes or preputial washes. Vigorous scraping of the preputial mucosa to obtain as much smegma as possible (Eaglesome & Garcia 1992) is the traditional method of collection. Stoessel and Haberkorn (1978) suggested that thorough scraping of the prepuce was needed to diagnose the presence of trichomonads, but Oosthuizen (1999) reported a very high reliability of preputial washings (using about 50 mL of phosphate-buffered saline or lactate Ringer's solution) collected from heavily sedated bulls. The bull should be allowed a period of 5 to 10 days of sexual rest before sampling so that the number of trichomonads can increase. The most reliable material for diagnosis in infected herds is preputial or vaginal washings or scrapings (OIE 2017e).

Whatever the source of the material that might contain trichomonads, it should be handled with care as the organism is fragile and degenerates very rapidly after death. Hence unless samples are handled properly, the organism may be absent by the time the samples are examined. Various media can be used for culture, including:

- Clausen's medium (Eaglesome & Garcia 1992);
- Diamond's medium (Diamond 1983); and
- InPouch TF system (Biomed Diagnostics Ltd) (Borchardt et al. 1992).

Organisms are visualised after culture. It is recommended that samples are transported to the laboratory in a Diamond's or InPouch medium rather than in a simple buffer. Temperature should be maintained between 22°C and 37°C. Culture systems using modified Diamond's medium and the InPouch TF have been considered as a 'gold standard' for the diagnosis of trichomonosis (Rae & Crews 2006).

The InPouch TF system for field culture consists of a clear flexible plastic pouch with two compartments. The upper compartment contains special medium into which the sample is introduced. Field samples for direct inoculation into the culture pouch would normally be collected by the preputial scraping technique. After mixing, the medium is forced into the lower compartment, and the pouch is then sealed and incubated at 37°C. Microscopic examination for trichomonads can be done directly through the plastic pouch. A preliminary diagnosis of trichomoniasis is made

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by observing motile trichomonads at 200x to 400x magnification. When present, the trichomonads are usually found in the corners and near the bottom of the lower or incubation compartment of the pouch. They are identifiable by their size, the presence of flagellae, and the 'undulating membrane'. For samples collected from bulls, the sensitivity of the InPouch system has been estimated at between 84% and 96%, and that of Diamond's medium as 78% to 99% (OIE 2017e). Specificity approaches 100%. Hence a proportion of infected bulls will not be detected at the first examination (Schonmann et al. 1994), so a second or third examination may be required to ensure that a bull really is negative.

PCR methods have recently been developed for the diagnosis of trichomonosis, which can differentiate between *T. foetus* and faecal trichomonads that contaminate the bovine preputial cavity (Campero et al. 2003, Dufernez et al. 2007), can be used to confirm the identification of *T. foetus* after initial culture (Parker et al. 2001, Grahn et al. 2005). PCR can be used for direct diagnosis of trichomonosis in field samples (Felleisen et al. 1998) with much higher sensitivity than using culture alone (McMillen & Lew 2006). However, samples must be assessed soon after collection: sensitivity declines rapidly with storage (Mukhufhi et al. 2003). It is considered that specificity and sensitivity of PCR are similar to those of the InPouch method (OIE 2017e).

Treatment and Control

Control can be attempted by:

- Eliminating bulls and replacing natural service by AI;
- Active management of groups of cows and use of bulls; or
- Treatment and/or vaccination of cows and bulls.

Artificial Insemination

Control through AI is based upon the assumption that recovery in the female is spontaneous and that infection of healthy animals cannot occur if natural service is replaced by AI using semen from non-infected bulls. Of all of the available methods, the elimination of bulls from the herd and the use of AI with uncontaminated semen is by far the most effective and efficient means of control. The method does require that cows should be bred exclusively by AI throughout at least one, and preferably, two seasons. Pregnancy rates to AI are likely to be poor during the initial period of its introduction because many of the cows may still be infected.

Group Management

Many different ideas have been suggested as ways of managing trichomonad-infected herds without resorting to the total use of AI. Most of these are similar to the control methods for bovine venereal campylobacteriosis (see earlier in this chapter).

An alternative strategy relies upon the limitation of effects of the disease by only using young bulls for breeding. It is argued that, because 2-year-old bulls are relatively resistant to infection, their use in breeding will result in less spread of the disease than occurs with older bulls. However, reliance upon the resistance of young bulls is unlikely to result in elimination of infection (Christensen et al. 1977), although their use may well help to reduce the level of infection that is present.

Treatment

As a general principle, carrier bulls should be culled because infection persists indefinitely. Bulls can potentially be treated with topical substances (e.g., iodine-based compounds, acriflavine, and imidazoles), but success rates are variable, elimination of infection is not reliable, and the application of such substances is anything but straightforward. Alternatively, dimetridazole or metronidazole can be potentially be given orally or intravenously. They have unpleasant side effects but are relatively effective. Ipronidazole can be used but has to be preceded by the use of broad spectrum antibiotics to kill non-specific bacteria in the prepuce that break down the imidazole (Skirrow et al. 1985). Resistance to the entire group of imidazoles is easily induced by the use of subtherapeutic doses. Unfortunately, none of these therapeutic substances is licensed for use in cattle in the UK or US. A newer antibiotic, trichostatin, has been found to be effective against *T. foetus in vitro* and *in vivo* (Otoguro et al. 1988). Even when treatment of individual animals is effective, it has no effect upon the presence of disease in the herd unless other steps are taken to ensure its eradication.

Vaccination

Many attempts have been made to develop a vaccine against *T. foetus.* Initial work used killed trichomonads in a mineral oil adjuvant (Clarke et al. 1983b), which helped eliminate infection from bulls. However, most development has been based upon fragmented cells or isolated membrane fractions, which stimulate a significant antibody response (Schnackel et al. 1990). These too have helped prevent and/or eliminate infection in cows and bulls (Kvasnicka et al. 1989, Hall et al. 1993, Hudson et al. 1993a, b).

In the US, a vaccine against *T. foetus* is available (TrichGuard; Fort Dodge). It requires two subcutaneous injections 2 to 4 weeks apart with the last injection given 4 weeks before the beginning of the breeding season (Rae & Crews 2006). Each subsequent year, all cows should receive a booster injection 4 weeks before the beginning of the breeding season. The vaccine does not prevent infection or disease but reduces the incidence and duration of infection of cows after service by an infected bull (BonDurant 1997). In experimentally infected heifers, TrichGuard increased the proportion of animals that conceived and that gave birth to a live calf (Edmondson et al. 2017) However, as the vaccine does not completely protect, it can only be used as an adjunct to other control or prevention methods (Cortese 1999). Curiously, although early studies in Australia suggested that vaccination conferred protection upon bulls, more recent American studies have found that vaccination has little effect upon either the incidence or the duration of infection in the male (Cortese 1999). However, despite these evidences for the effectiveness of killed whole-cell *T. foetus* vaccines, when Baltzell et al. (2013) undertook a meta-analysis of relevant literature, they found only moderate evidence for its efficacy: the relative risk for infection was 0.89, for failure to conceive was 0.8, for abortion risk was 0.57, and the data were too imprecise to calculate an effect upon duration of infection. It was not possible to calculate an effect upon infection of bulls.

Neosporosis

Neospora caninum was first discovered as a protozoan parasite that causes encephalomyelitis of dogs (Dubey et al. 1988). Neosporosis is now recognised as a significant cause of bovine abortion in most of the major cattle-producing regions of the world. It has been recorded in the UK, the US (Dubey & Lindsay 1996), Canada (Alves et al. 1996), Argentina (Campero et al. 1998), South Africa (Jardine & Last 1995), Zimbabwe (Wells 1996), Australia (Obendorf et al. 1995), New Zealand (Thornton et al. 1991), and many other countries. In 1999 Tenter and Shirley suggested that *N. caninum* was responsible for 6000 abortions per annum in the UK. Reichel

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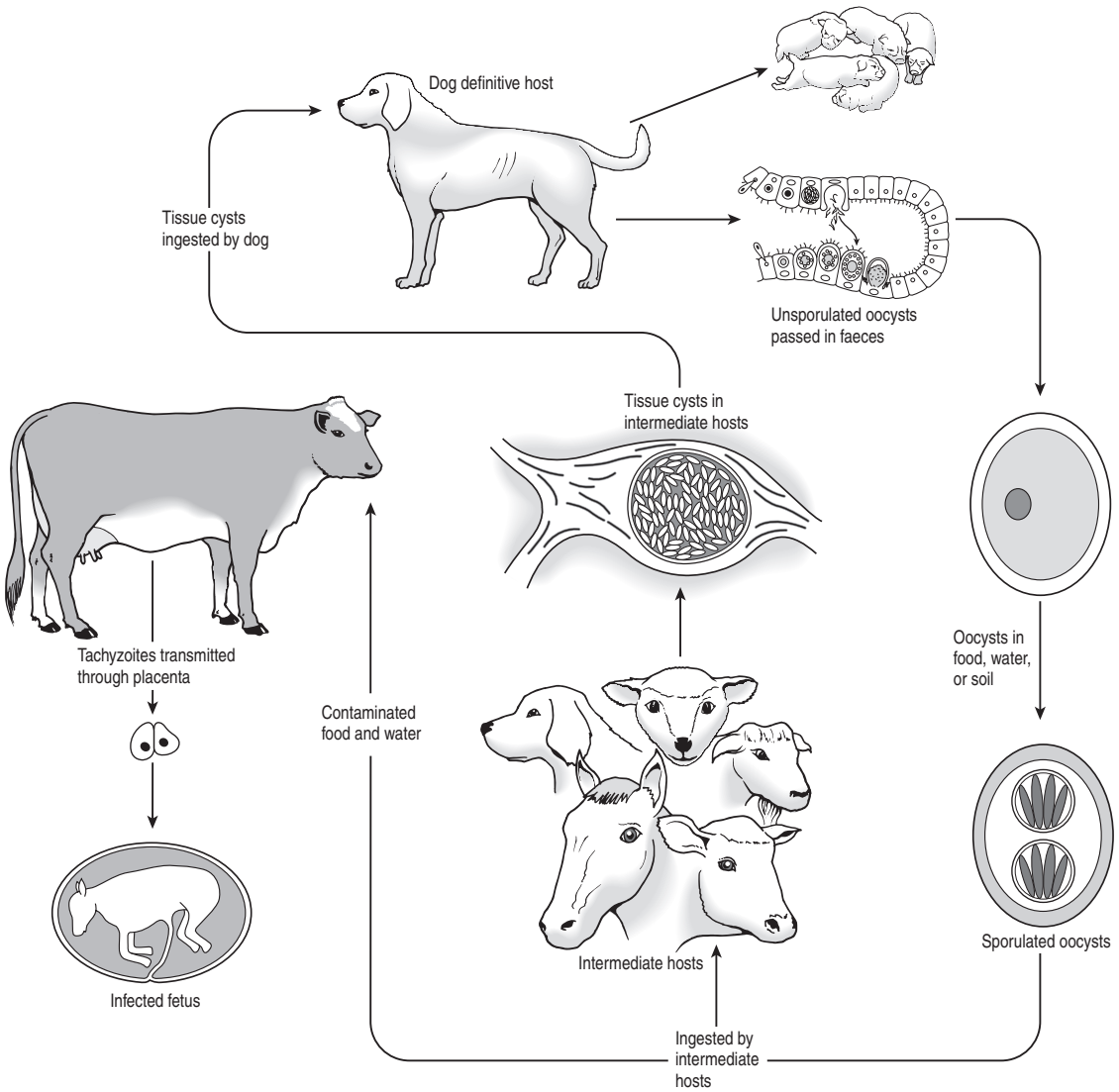

• **Fig. 24.7** Life cycle of *Neospora caninum*.

et al. (2013) undertook a meta-analysis of published sources on the economic effect of the disease: they estimated a median cost to dairy farms in the UK of US$700 to $2100; in the US of US$3700 to $16,100; in Australia of US$2500 to $18,000; and in New Zealand of US$4500 to $68,000.

Aetiology and Pathogenesis

The dog is both the definitive host and an intermediate host for the parasite (McAllister et al. 1998). The life cycle consists of three infectious stages: tachyzoites, tissue cysts, and oocysts (Dubey 2005) (Fig. 24.7). After ingestion of tissue cysts, dogs may pass small numbers of unsporulated oocysts in their faeces, which may contaminate feed or water of grazing animals and which are reasonably resistant in the environment. It is likely that other canids (e.g., foxes, coyotes) and scavenger species may also be definitive hosts (Wapenaar et al. 2006). When sporulated oocysts are ingested by an intermediate host (dogs, grazing species, and birds), sporozoites are liberated into the intestinal tract, which then penetrate cells to become tachyzoites. These divide rapidly, causing tissue damage, and then spread to a variety of tissues including neural cells, macrophages, fibroblasts, vascular endothelial cells, hepatocytes, and placenta. Thereafter, the parasite forms bradyzoites (tissue cysts), predominantly in neural tissue (Antony & Williamson 2003, Weston 2008). These tissue cysts can subsequently be eaten by a definitive host, thereby perpetuating the cycle. Transmission to the definitive host occurs mainly after ingestion of fetal or placental tissues infected with tissue cysts.

Vertical transmission via the placenta to the fetus is considered to be the main route by which cattle became infected (Anderson et al. 2000). Marugan-Hernandez (2017) summarised the routes of vertical transmission as follows:

- Transplacental transmission result from an endogenous or exogenous source of infection.
- Endogenous transmission occurs when there is reactivation of tissue cysts in a previously/persistently infected animal and is associated with a pattern of sporadic abortions and reproductive failure in the herd.
- Exogenous infection occurs after a primary horizontal infection (i.e., oocysts are ingested by a pregnant animal) and is associated with an outbreak of abortions, usually in a naïve herd.

There is no direct evidence for horizontal transmission from cow to cow (Anderson et al. 1997). However, epidemiological evidence

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from abortion storms suggests a point source of infection (McAllister et al. 2000). Furthermore, de Magalhaes et al. (2014) concluded from studies of seroconversion rates in cattle that horizontal transmission could occur in approximately 3% of animals. Routes of horizontal infection could include colostrum, fetal membranes, and fluids from infected cows or oocyst-contaminated feed. The presence of *N. caninum* in semen also raised the possibility of this route of transmission (Piagentini et al. 2012). Likewise, horizontal transmission could occur if cattle ingest tachyzoite-contaminated pasture and have concurrent oral lesions (such as occur when permanent incisors are erupting) that would allow haematogenous spread. None of these routes of infection has been convincingly proven, although there is a clear association between the presence of *Neospora* infection in farm dogs and the risk of abortion in dairy cows (Bartels et al. 1999, Wouda et al. 1999). On the other hand, some farms have a high proportion of adult cattle that seroconvert without any increase in the occurrence of abortions.

Clinical Signs

Neospora caninum infection causes abortion at any time after 3 months of gestation, although abortions are most common in the fifth to seventh months. Infection of an immunologically competent fetus most commonly results in the birth of a live, congenitally infected calf, although stillbirths also occur. Thus fetuses may be resorbed, mummified, autolysed, stillborn, born alive with clinical signs, or born clinically normal but chronically infected (Dubey 2005). A congenitally infected heifer calf is capable of vertically transmitting the infection to the next generation when she becomes pregnant, thus maintaining infection within a herd. Repeat abortion may occur in some infected animals. Abortions may present as:

- An abortion 'storm', in which up to 40% of a herd may abort in one season (Anderson et al. 1995). There appears to be two circumstances in which this occurs:
  - simultaneous exposure cattle to infective oocysts from a definitive host, as described previously by Marugan-Hernandez (2017); and
  - exposure to another infectious agent (e.g., BVD virus) or factor that suppresses immunity (Antony & Williamson 2003)
- Sporadic abortions in endemically infected herds. Abortions can be an ongoing problem, with an abortion rate that is high but not catastrophic. Other herds experience clusters of abortions over a period of 2 to 3 months, presumably reflecting the timing of exposure to the parasite (Weston 2008).

Patterns of abortion also differ between congenitally infected animals and those that become infected after birth. Congenitally infected animals are permanently infected and are at a high risk of abortion, particularly in their first gestation. Thereafter, they are more likely to produce live calves, although there is a very high probability (75%–90%) of their offspring also being congenitally infected. Animals infected by horizontal transmission may abort or may produce uninfected or congenitally infected calves depending on their immune status and the stage of gestation at which they were infected.

Calves that are congenitally infected with *N. caninum* may be underweight and/or have neurological signs, including ataxia, impaired proprioception, or an inability to rise. Neurogenic flexion or hyperextension of the limbs may occur (Barr et al. 1993). Exophthalmia, asymmetry of the eyes, and hydrocephalus have also been reported (Dubey 2005). However, most calves that are congenitally infected are clinically normal (Thornton et al. 1991).

Diagnosis

Abortion due to *N. caninum* may need to be differentiated from other protozoal agents such as *Toxoplasma gondii* or *Sarcocystis.* Abortion diagnosis is made by a combination of serology, with immunohistochemistry and histopathology of aborted fetuses.

Fetuses aborted due to *N. caninum* infection are characteristically moderately to severely autolysed (Abbitt & Rae 2007). Diagnosis of *N. caninum* infection is relatively straightforward, using serology and/or demonstration of tissue cysts. The organism can cause lesions in several organs, of which fetal brain is the most consistently affected. Typically, there is a focal encephalitis characterised by necrosis and non-suppurative inflammation. Because most aborted fetuses undergo rapid autolysis, even semiliquid brain tissue should be fixed in 10% buffered neutral formalin for histological examination. In calves that are congenitally infected with *N caninum,* one of the specimens of choice for diagnosis is brain and spinal cord from dead calves. Non-suppurative lesions are also present in the myocardium, skeletal muscle, and, occasionally, the liver and lung (Weston 2008). Non-suppurative lesions are also present in the placenta, which often presents with multifocal necrotic foci within the cotyledons combined with intact intercotyledonary areas. Inflammatory infiltrates begin in the maternal caruncles and then extend to the fetal cotyledon, with the appearance of areas of haemorrhage and necrosis (Marugan-Hernandez 2017). Confirmation of a presumptive diagnosis on the basis of histology requires demonstration of the presence of tissue cysts by either immunocytochemistry or serology.

PCR methods are available for the diagnosis of the presence of *N. caninum* in both fetal tissues (Reitt et al. 2007) and colostrum (Moskwa et al. 2007). However, McInnes et al. (2006) noted that, although the presence of *N. caninum* can be demonstrated by PCR, neither the presence nor absence of antibodies or DNA could unequivocally support or exclude it as the cause of abortion. Hence they concluded that additional criteria are required for a positive diagnosis of neosporosis as the cause of an abortion or that it is best used to confirm a presumptive diagnosis made on clinical signs and pathological lesions.

There are several serological tests that can be used to detect neosporosis in dairy herds, including immunofluorescent antibody (IFAT) and ELISA tests. IFAT titres of more than 1:200 are often regarded as being indicative of previous infection, whereas a titre of more than 1:2000 is indicative that a recent abortion was due to *N. caninum.* However, because of the widespread prevalence of seropositive cows, a positive result does not necessarily indicate infection at the time of testing, only that the cow had been exposed to the disease at some previous time. Moreover, non-aborting cows have been reported with titres of more than 1:4000, although titres can have dropped significantly in cows in the interval between infection and abortion.

Fetal serology can definitively demonstrate the presence of infection with *N. caninum,* although the absence of a positive response is not a definitive indication that infection did not occur, especially in early gestation fetuses that have not become immunologically competent. In live calves that are congenitally infected with *N. caninum,* precolostral serum is the specimen of choice for diagnosis.

Prevention and Control

The vertical transmission of *N. caninum* and limited understanding of the means whereby horizontal transmission occurs makes control difficult. There are three main strategies that are in use: management of breeding to reduce vertical transmission; testing and culling affected animals; and reduction of the canid-ruminant

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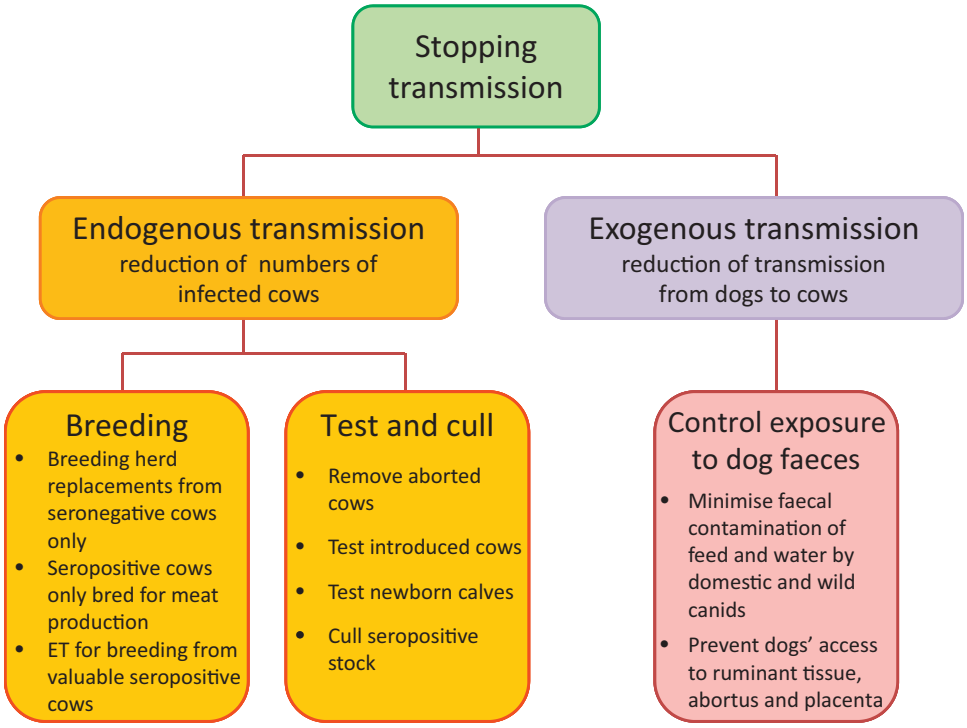

• **Fig. 24.8** Approaches to the control of *N. caninum* infections and abortions. Pathways showing ways for the reduction of the number of *N. caninum*-infected cows, for managing exposure to dogs and canids, and for preventing transmission of *N. caninum* to cattle. (Based on Reichel et al. 2014 and used with permission.)

life cycle (Fig. 24.8). None are fully effective, but depending on the circumstances of the herd, all may help to reduce the incidence of abortions. Vaccination, the potential fourth method of control, is not currently feasible. There is no effective means of treatment.

If vertical transmission (i.e., from one generation of cattle to the next) can be prevented, future generations of cattle will be uninfected and should have a reduced risk of abortion, provided that further exposure to oocysts can be prevented (Reichel et al. 2014). Thus cows that are seropositive, which are likely to be infected, will produce daughters that are also infected and are at risk for abortion at least in their first pregnancy. Not breeding herd replacement from such animals or breeding them only for production of slaughter-generation animals will preclude such vertical transmission. Avoiding bringing seropositive animals into the herd (whether home-bred seropositive calves or purchased animals) will also reduce the risk of vertical transmission. When the proportion of infected animals in a herd is relatively small, carriers can be identified by serology and culled (Reichel & Ellis 2002), thereby preventing vertical transmission. If calves are required from infected cows (e.g., when valuable genetics need to be conserved), these can be derived by embryo transfer, provided uninfected recipients are available.

Measures that can be taken to limit horizontal spread include:

- Ensuring that cattle do not have access to contaminated feed or water;
- Removal and disposal of abortus material and afterbirths immediately after birth; and
- Preventing dogs from having access to cattle feed, effective rodent control, and preventing wild carnivores from having access to abortus material.

A killed *N. caninum* vaccine, based on inactivated tachyzoites, has been developed, but does not appear to stimulate protective immunity. Use of the vaccine reduced the incidence of abortions but did not prevent them (Choromanski & Block 2000, Romero et al. 2004). One of the main problems with vaccination is that cows that are apparently immune to the disease (i.e., are seropositive) can still undergo repeat abortions. Vaccination appears to be more effective at preventing abortion due to new infection after the ingestion of *Neospora* oocysts than it is at preventing abortion in animals with congenital infection (Trees & Williams 2003, Dubey 2005). Based upon experience of toxoplasmosis in sheep, Marugan-Hernandez (2017) considered that live vaccination was likely to give the best results, so long as a low virulence isolate with low capacity to persist in the host can be identified.

Sarcocystis spp.

Sarcocystis is a very rare cause of bovine abortion (Abbitt & Rae 2007). Protozoa of the *Sarcocystis* genus have a two-host life cycle. The definitive host, which is a carnivore, sheds infectious sporocysts in its faeces. The intermediate host ingests the sporocysts, which develop into sarcocysts in the muscle of the host. Of the three species of *Sarcocystis* that occur in cattle (Markus et al. 2004), *S. cruzi* appears to be able to cause abortion. Clinically, abortions are indistinguishable from those caused by *Neospora;* differentiation depends on immunohistochemistry or PCR.

Viral Agents

Bovine Viral Diarrhoea

Bovine viral diarrhoea (BVD) virus was initially recognised as a cause of diarrhoea during the 1940s. Although it was originally considered to be a simple virus-induced diarrhoea, more recent

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understanding of the infection has shown that it also causes infertility and abortion. Worldwide, BVD virus is a major cause of abortions, resulting in both sporadic abortions and abortion storms.

Aetiology and Pathogenesis

Bovine viral diarrhoea virus is a pestivirus of the Flaviviridae family, which also contains classical swine fever and border disease of sheep. There are two genotypes of BVD virus: BVDV-1, which has a worldwide distribution, and BVDV-2, which is largely restricted to the US. Disease associated with BVDV-2 is generally much more severe than that resulting from BVDV-1. BVDV is also divided into non-cytopathic strains and cytopathic strains, based upon their effects upon cells in tissue culture. Both the non-cytopathic and cytopathic isolate exist within each of the two BVDV genotypes. There is strong evidence that the non-cytopathic biotype mutates to the cytopathic biotype in persistently infected (PI) animals.

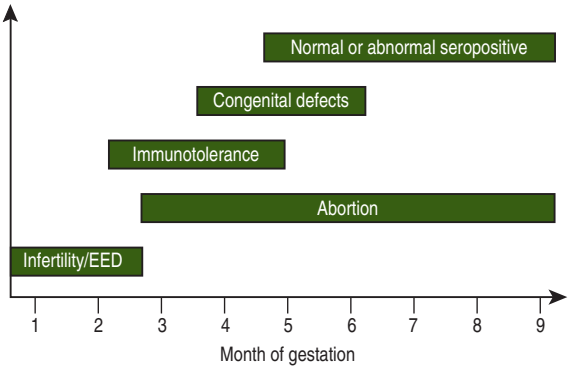
The main route of infection of naïve animals is via respiratory secretions, but it can also spread in uterine secretions and abortuses, urine, milk, semen, faeces, and saliva. BVDV can also be transmitted through virus-contaminated embryos (Avery et al. 1993). It appears that relatively close contact, including nose-to-nose and sexual contact, is required for infection to occur. Acutely infected animals, flies, aerosolised virus, and contaminated pens or veterinary equipment have also been implicated in transmission (Lanyon et al. 2014). Infection of naïve animals results in a transient viraemia, which may be accompanied by pyrexia, mild inappetence and diarrhoea, depression, and a period of immunosuppression, from which animals recover in a few days. Some affected animals may develop oculonasal discharges, salvation, and oral erosions, and, occasionally, there may be more severe disease. Most animals, however, show little or no clinical evidence of infection (Barr & Anderson 1993, Radostits et al. 2007). In pregnant females, transplacental infection of the fetus occurs during the period of viraemia.

Depending on the time of its occurrence, infection of the fetus can result in early embryonic death, abortion, birth of live or stillborn calves with congenital defects, birth of live calves with a persistent BVDV infection, or birth of seropositive calves that are immune to the virus. Such persistently infected (PI) animals are the main means of spread of the virus and are considered to be the main means by which BVDV infection is maintained in populations of cattle (Bolin 1990a).

Mutation of the non-cytopathic biotype in PI animals results in the development of mucosal disease. Mucosal disease is generally considered to be a sporadic condition, affecting single animals or small numbers of animals in a sequential manner. However, if there are a large number of PI animals in a herd (as might occur if infection was introduced to a herd at the susceptible stage of pregnancy), an outbreak of disease can occur when animals with mucosal disease excrete cytopathic biotype virus that infects the other PI animals. In this circumstance, mucosal disease appears to behave as a simple infectious disease. It is generally considered that cytopathic biotype cannot cause transplacental infection, nor can it cause PI animals.

Clinical Signs

Infection with BVDV causes reproductive failure in naïve and PI females but rarely in seropositive animals. The introduction of BVDV to susceptible breeding females around the time of insemination and during the embryonic and early- to midfetal period can result in a range of reproductive disorders and neonatal diseases (Fig. 24.9). In addition, acute infection with BVDV can allow other infections (notable *Neospora caninum*) to undergo recrudescence.



• **Fig. 24.9** Potential clinical effects upon reproduction in cattle after infection with bovine viral diarrhoea virus at different stages of gestation (EED, early embryonic death). (Redrawn from Grooms and Bolin 2005.)

Infection From Before Mating to Day 30 of Gestation

Infection can occur at mating if cows are bred by a PI bull or an animal that is transiently infected with BVDV (Kirkland et al. 1991), or if inseminated with semen that is contaminated with the virus (Virakula et al. 1993). Infection with BVDV at or around the time of breeding can have a significant effect on reproductive performance: affected animals are less likely to become pregnant (Houe et al. 1993, Grooms 2004) due to conception failure and impaired early embryonic development (McGowan et al. 1993). This in turn is due to effects upon follicle and oocyte function, the magnitude of the LH surge (Ssentongo et al. 1980, Grooms et al. 1998), and the uterine environment. Oocytes can be infected with BVDV, which may explain why calves born to PI cows are always PI themselves (Lanyon et al. 2014). Pregnancy failure may be manifest as a low non-return rate or as irregular, prolonged returns to oestrus. The birth of PI calves after insemination of cows with semen infected with BVD virus has also been reported (Meyling & Jensen 1988).

Infection Between Days 30 and 150 of Gestation

Infection of the fetus in this period of gestation can lead to:

- Fetal resorption (Days 30–40), mummification, or abortion;
- Immunotolerance and the birth of PI calves (Days 30–150); and
- Congenital defects, mainly of the central nervous and ocular systems (Days 80–150).

Fetal deaths after BVDV infection of susceptible dams can occur at any point of gestation, although they are most common during the first trimester. Depending on the time of infection, fetal resorption, mummification, or expulsion can occur. Expulsion of the fetus may occur from days up to several months after fetal infection (Bolin 1990a).

Fetuses that survive infection with non-cytopathic BVDV between Days 18 and 125 of gestation (McClurkin et al. 1984) are immunotolerant to the virus and subsequently become persistently infected with BVD virus. Most infections that result in the development of PI calves occur before Day 75 (Roeder et al. 1986) but, although immunotolerance is uncommon after Day 100, it can occur until Day 125 (Grooms 2004).

Infection between Days 80 and 150 of gestation can result in the birth of calves with congenital abnormalities, predominantly of the central nervous system and eyes (Table 24.4). Typically, there is a time interval of between several days and 2 months between infection with BVD virus and abortion (Bolin 1990a).

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TABLE 24.4 Congenital abnormalities associated with bovine viral diarrhoea virus infection during midgestation

Nervous System	Eye	Other Systems
Cerebellar hypoplasia	Cataracts	Long bone deformities
Hypomyelinogenesis	Microphthalmia	Stunted growth
Hydrocephalus	Retinal atrophy	Brachygnathias
Microcephalus	Optic neuritis	Hair abnormalities, alopecia

Infection in Late Gestation

Irrespective of the biotype, infections of the fetus in the later stages of pregnancy that do not cause abortion will lead to the birth of an immune calf, because the fetus can develop a measurable antibody response to the organism by 5 to 6 months of gestation (Bolin 1990b).

The Trojan Cow

A non-PI cow that is carrying a PI fetus can be considered a 'Trojan cow' (Lanyon et al. 2014). The cow appears immune to BVDV and is healthy, but the PI calf that she is carrying will shed large quantities of BVD virus once it is born. Previous observations have shown that Trojan cows have antibody titres during mid to late pregnancy that are significantly higher than those of seropositive cows carrying normal calves (Brownlie et al. 1989, Lindberg & Alenius 1999). This high antibody titre is very probably a result of the continual antigenic challenge of the cow.

Abortion

Abortions can occur at any stage of gestation. Most losses occur before the third trimester, as the fetus is increasingly able to mount an effective immune response against the virus as gestation advances, but some abortions in the third trimester of pregnancy have also been attributed to BVD virus (Moennig & Leiss 1995, Grooms & Bolin 2005).

Fetuses aborting from BVDV infection may be expelled autolysed, mummified, or in fresh condition at various stages of pregnancy. However, because of the relatively long interval between infection and abortion, the fetus is often severely autolysed. There are no pathognomonic fetal lesions associated with BVDV infection but, if the fetus is reasonably fresh, there may be histological evidence of dermatitis, meningitis, destruction of the cerebellar cortex, and bronchiolitis.

Diagnosis

Aborted or Congenitally Malformed Fetus

Demonstration of BVDV in any tissues of affected fetuses or calves by virus isolation, immunohistochemistry, demonstration of BVDV antigen in fetal fluid or skin by ELISA, or PCR testing of fetal fluid would confirm the presence of BVDV infection (Lanyon et al. 2014). When infection is acquired after Day 150 to 180 of gestation, the fetus is able to mount an immune response, will eliminate the virus, and will be born BVDV antibody-positive, antigen-negative (Hansen et al. 2010). Neonatal serology must be undertaken in precolostral animals to avoid confusion with maternal antibodies. Virus can be recovered from fetal lymphoid tissues (spleen, thymus, and ileum), lung, or liver by virus isolation, PCR, or immunohistochemistry. It is preferable to submit multiple fetuses until the virus is isolated (Grooms & Bolin 2005).

Herd Infection

The presence of active BVDV infection in a herd can be inferred from clinical signs, the presence of PI animals and monitoring programmes. Bulk milk monitoring is an effective means of detecting the presence of a PI animal among the lactating cows, owing to the very large amount of virus that such animals produce. Bulk milk testing can be undertaken by ELISA (Pritchard 2001, Thobokwe et al. 2004) or, more usually now, by PCR (Renshaw et al. 2000, Pritchard 2006).

Serology is relatively difficult to interpret, as the virus is widespread and antibody titres are long lasting, with the result that high titres can be indicative of historical, rather than current, infection. It is also difficult to differentiate between natural infection and vaccination titres (Grooms & Bolin 2005). However, serology of young (~9 months old), unvaccinated animals that are used as 'sentinel animals' is a useful means of monitoring the presence of active infection. The presence of antibody titres in these unvaccinated cattle indicates recent virus exposure and is a strong indicator that BVDV is present on the farm (Houe 1992, Pillars & Grooms 2002). Likewise, antibodies in the serum of precolostral calves are also indicative that active infection occurred during their gestation. Booth and Brownlie (2016) noted that bulk milk antibody tests provide an initial, rapid, and cost effective assessment of the BVDV status of dairy cattle. It is generally accepted that high antibody levels correlate with a high probability of the presence of a PI animal. They also considered that antibody tests on young animals are valuable because such cattle will be seronegative if unexposed; the presence of antibodies demonstrating an active immune response (in contrast to maternal colostral antibodies) means that they have been infected within the 9 months of their life. That, in turn, would indicate the presence of active infection on the farm.

Demonstrating the presence of PI animals can be used to confirm that active infection with bovine viral diarrhoea is occurring. Samples for the diagnosis of PI animals include (Kelling 2007):

- Virus isolation (blood; buffy coat). Samples should not be collected from calves that have maternal antibody. It may be necessary to differentiate PI animals from those with acute, transient, infections. High sensitivity and specificity;
- Immunocytochemistry (ear notch biopsies). Can be used on any animals. High sensitivity and specificity;
- PCR (blood, serum, skin biopsies, ear notch). High sensitivity, but specificity can be affected by non-specific reactions; and
- ELISA for viral antigen (blood). High specificity and sensitivity (Hill et al. 2007) also used to differentiate between persistent and transient infections.

Control

Control should be aimed at preventing entry of BVD virus to naïve herds and preventing the spread of the virus in infected herds.

Biosecurity measures to prevent the entry of infection include maintaining a closed herd, testing all incoming animals for the presence of BVD virus/viral antigen (i.e., identifying persistently or transiently infected animals), and ensuring no pregnant animal that might be carrying a PI calf is brought on to the farm. Bulls that are purchased and brought on to the farm from outside sources pose a significant risk of infection to herds that are otherwise closed. It is possible that the disease may be transmitted from other ruminants, so maintaining separation between such animals and susceptible cattle is recommended. It is possible that semen could be infected with BVD virus, but disease control at bull studs should preclude this.

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TABLE 24.5 Bulk milk BVDV antibodies: likely significance and suggested actions

Antibody Level	Indicative Proportion of Seropositive Cows (%)	Herd Status		Possible Further Action
Low	< 5	Naïve		Test young stock to ensure continued lack of exposure
Intermediate	5–25	Low exposure	May indicate historical or acute infection	Test 'sentinel' animals and/or first lactation heifers. If antibody – ve, continue monitoring. If + ve, consider implementing control measures
	25–65	Moderate exposure		
High	> 65	High level of exposure; recent exposure to infection, with a high probability of persistently infected (PI) animals		Implement eradication policies to remove PI animals. Vaccination should be considered

Based on Brownlie (2005).

Various strategies exist for controlling infection in affected herds, depending on the level of disease that is present in the herd (Table 24.5). The use of a PI animal as an 'endogenous vaccinator' has been recommended in the past, but this is now very strongly discouraged (Brownlie 2005). It is now considered preferable to identify and remove PI animals because these are the main source of infection. This will not prevent spread by continued transient infections, so vaccination may also be required.

Modified live and killed vaccines have been developed for control of BVD. Advantages of modified live vaccines are a long duration of action and interstrain crossreactivity. Conversely, they may cause immunosuppression and have been associated with fetal abnormalities (Kelling 2007). Killed vaccines neither have immunosuppressive effect nor have they been associated with fetal abnormalities, but they may not protect against abortion when animals are exposed to high levels of infection (Laven et al. 2003, Packianathan et al. 2017). The whole herd may be vaccinated or just the heifers (maiden and primiparous animals), depending on circumstances (Brownlie 2005). It has been suggested that, if live vaccine is available, cattle should be vaccinated with killed vaccine before being given the live vaccine. Eradication regimes are being implemented in many countries in which bovine viral diarrhoea is endemic. These schemes are beyond the scope of the present text.

Bovine Herpesvirus 1

Bovine herpesvirus 1 (BoHV-1) has a worldwide distribution and is associated with infectious bovine rhinotracheitis, infectious pustular vulvovaginitis (IPV), balanoposthitis, abortion and infertility. There are three subtypes of BoHV-1 (Babuik et al. 2004), which are associated with different diseases (Table 24.6). Within subtypes, there are strains that cause different levels of severity of disease.

Aetiology and Pathogenesis

Infection occurs by the respiratory route and, in the case of IPV, by the venereal route. It may also be transmitted through infected bedding, sniffing of the vulva and perineum of infected animals, and via contaminated semen. Abortus material is also a significant source of infection.

Infection can result in clinical presentations of disease of varying degrees of severity. Animals may become asymptomatic latent carriers directly after infection or after recovery from clinical illness. Latent infections are maintained in the trigeminal and sacral ganglia.

Acute signs of respiratory or genital disease appear after a latent period of 10 to 20 days, with respiratory symptoms generally being of short duration. Lesions associated with IPV may be present for a longer period – sometimes up to several weeks. Excretion of virus in respiratory or genital secretions continues for approximately 14 days. However, animals that become latent carriers may resume shedding of the virus periodically during times of stress (e.g., calving, transport) or after corticosteroid administration, at any time for the remainder of the animal's life.

TABLE 24.6 Disease syndromes associated with different subtypes of Bovine Herpes Virus (BoHV)-1

	SYNDROME		
Type	Infectious Rhinotracheitis	Infectious Pustular Vulvovaginitis/ Balanoposthitis	Abortion
BHV-1.1	+	-	+
BHV-1.2a	+	+	+
BHV-1.2b	+	+	-

The interval between infection with an abortifacient strain and expulsion of the fetus is much more variable, ranging from a few days to full-term delivery of a stillborn or affected calf (Miller et al. 1991). It has been suggested that the duration and variability of the interval between infection of the dam and abortion of the fetus represents a period in which the virus resides in the placenta without infecting the fetus itself (Kendrick 1971).

Clinical Signs

The genital form of the disease may present without other symptoms or be accompanied by respiratory disease. It is unusual for IPV and abortions to present together.

Infectious Pustular Vulvovaginitis

The onset of vulvovaginitis is sudden and acute. Signs appear 24 to 48 hours after venereal transmission. Heifers tend to be more severely affected than cows. The vulvar labia become swollen and tender and, in light-skinned animals, deeply congested. This is quickly followed by the development of numerous red vesicles on the mucosa. These may rapidly rupture or develop into pustules which give rise to haemorrhagic ulcers.

The quantity of vulvar discharge is variable, ranging from small quantities of exudate that adheres to the vulval and tail hairs, to a

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copious mucopurulent discharge. A speculum is useful to examine the vaginal mucosa, but because of the pain and discomfort, caudal epidural anaesthesia is worthwhile. The lesions are obviously painful because affected animals are restless, with swishing of the tail, and dysuric, with frequent urination and straining. There may be transient pyrexia and reduced milk yield, but the systemic effects are variable depending on the presence of respiratory problems. The acute phase of the disease will subside in about 10 to 14 days, but a few animals will display a persistent vulval discharge for several weeks.

When females show signs of IPV, the bull must be examined for the presence of lesions because, unlike the situation with most venereal diseases of cattle, the signs in the bull are dramatic (see Chapter 36).

Infertility

Infection with BoHV-1 around or after the time of breeding is associated with poor fertility. Insemination with infected semen results in poor pregnancy rates (Kendrick & McEntee 1967, Parsonson & Snowdon 1975). There are various contributors to this. First, Miller and van der Maaten (1984) showed that there is a localised endometritis that follows intrauterine inoculation of BoHV-1. Second, there are necroses and lymphoid proliferation in the parenchyma of the corpus luteum, as well as in non-luteal ovarian parenchyma, and there are necrotic follicles in the ovaries (Miller & van der Maaten 1986). Additionally, the presence of BoHV-1 in semen impedes binding of sperm to the zona pellucida, thereby reducing fertilisation rate (Tanghe et al. 2005). Intrauterine inoculation with BoHV-1 on Days 7 and 14 after breeding is associated with embryonic necrosis or the absence of a conceptus and a delayed return to oestrus, signifying that embryonic death had occurred. It appears that embryonic death is the result of a direct invasion of BoHV-1 into embryonic cells (Bowen et al. 1985, Miller & van der Maaten 1986, 1987). Progesterone production is reduced in response to BoHV-1 challenge, not only in the cycle in which the challenge occurs but also, in a significant proportion of animals, in at least one subsequent cycle. From these data, Chase et al. (2017) concluded that the corpus luteum is a major site of BoHV-1 pathology in situations in which infection occurred within 4 to 9 days after oestrus and that progesterone concentrations were decreased regardless of whether the animal had luteal lesions.

Abortion

Worldwide, BoHV-1 is a significant cause of bovine abortion. Kirkbride (1992) reported that, among nearly 9000 abortions that occurred between 1980 and 1990, BoHV-1 was responsible for 5.4% of incidents. Murray (1990) found IBR to be the causative agent in 13% of 149 calves that were aborted over 2 years in northwest England. Most abortions are sporadic, but abortion storms can occur (e.g., Tanyi et al. 1983). However, abortion is considered to be a less significant consequence of BoHV-1 infection in the UK and Europe than it is in North America (Caldow & Gray 2004). Only Subtype 1.2b, which does not cause abortion, is present in Australia and New Zealand. Respiratory disease is not always evident before abortions occur, although the length of time between infection and expulsion of the fetus can mean that earlier respiratory disease may be overlooked (Barr & Anderson 1993).

Abortions occur from 4 months of gestation to term, most commonly between the fourth and eighth months of gestation. Some calves are stillborn, and a few may be born alive but succumb subsequently. The effects of virus infection may be due to the strain of the virus: Miller et al. (1991) reported that infection of heifers at 25 to 27 weeks of gestation with Subtype 1.1 resulted in abortions 17 to 85 days later, but those given Subtype 1.2a delivered full-term calves, some of which had BoHV-1 neutralising antibodies in precolostral serum. On the other hand, the interval between the time when the fetus itself becomes infected (i.e., rather than the dam) and its expulsion is relatively short: Kelling (2007) suggested that it is no more than 7 days. Regardless, the interval between fetal infection and expulsion is sufficient for the fetus to become highly autolysed or, less commonly, mummified. Retention of the fetal membranes is a common sequel to abortion.

Diagnosis

The genital tract lesions of IPV are fairly characteristic of the disease but must be differentiated from granular vulvovaginitis due to *Ureaplasma* spp. and catarrhal vaginocervicitis. After the presence of genital lesions, vaginal swabs, preputial washings, and semen should be placed in virus transport medium. Paired serum samples should be taken from the affected cows.

A severely autolysed aborted fetus is highly suggestive of BoHV-1 infection. There is frequently a liquefactive necrosis of the whole of the kidney cortex with perirenal haemorrhagic oedema. Histologically, there is always focal necrosis of the liver, and in many cases there are necrotic lesions in the brain, lungs, spleen, adrenal cortex, and lymph nodes. There are characteristic virus inclusion bodies at the periphery of these necrotic lesions in fresh experimental cases, but, because of autolysis, they are not always demonstrable in field cases of abortion. Virus may be present in any fetal tissues and in the cotyledons (Kirkbride 1992). The best means of identifying BoHV-1 in an aborted fetus is though PCR of the fetal liver. Crook et al. (2012) compared IHC and PCR-based methods of diagnosis with routine diagnostic measures; the former identified 10 of 400 abortions as being due to BoHV-1, but routine methods only identified 2 of 400 cases.

Serology is of little value in establishing a diagnosis of BoHV-1 abortion in endemic regions, as maternal infection may precede abortion for up to 2 months. Hence paired serum samples are unlikely to demonstrate a postinfection rise in titres because, by the time abortion occurs, maternal antibody levels may have already peaked, and demonstrating a rise in specific antibody levels may no longer be possible (Borel et al. 2014). Serological examination at a herd level may be of some value. Kirkbride (1990) recommended collecting paired serum samples from at least 10 cows, which should reveal seroconversion or a fourfold increase in titres if BoHV-1 infection is active in the herd.

Control

Spontaneous recovery of the genital lesions will occur, and therefore treatment is not really necessary. However, the administration of emollient creams to the vulva, vagina, and penis may be useful. Vulval stenosis and penile/preputial adhesions and phimosis can occur during the healing phase (see Chapter 36). Infected animals should be isolated and natural service suspended. Vaccination, together with sound biosecurity measures, is the most effective way of controlling the disease. A number of vaccines are available, including modified live and killed vaccines. Heifers should be vaccinated after 6 months of age and before their first service; thereafter, annual vaccination is preferable.

Pregnant animals should only be vaccinated with a killed vaccine, as modified live vaccines can potentially produce all of the effects of natural BoHV-1 infections upon the reproductive system (Miller 1991). Abortions have also been reported after vaccination with

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a modified live vaccine (Kelling et al. 1973), but there is disagreement regarding whether this is a consequence of the vaccine per se or to other strains of the virus. Ostertag-Hill et al. (2015) examined BoHV-1 isolated from animals that had aborted after vaccination, with the finding that many disease isolates had genetic differences from the vaccine strain and that some animals had a number of different genotypes present. On the other hand, Fulton et al. (2015) and Chase et al. (2017) considered that a significant proportion of fetal losses originated from vaccine use. In contradiction to both of these studies, the meta-analysis of Newcomer et al. (2017) of the effects of vaccination against BoHV-1 upon abortions showed that the relative risk of abortion in animals that had had a modified live vaccine was 0.42, whereas it was 0.37 in those that had had an inactivated vaccine. When only field challenges were included, vaccination reduced the relative risk of abortion to 0.64. Interestingly, in view of the potential immunosuppressive effects of BVDV, Walz et al. (2017) showed that prebreeding vaccination and annual revaccination with modified live BoHV-1 and BVDV vaccine conferred significant protection against this potentiating effect of one virus upon the other, although when vaccinated heifers were inoculated with high doses of BoHV-1 virus and exposed to an animal persistently infected with BVDV, it did not entirely preclude abortions.

Taken together, these data support the earlier recommendation of Kelling (2007):

- Beef heifers should be vaccinated before the start of the breeding season.
- Dairy heifers should be vaccinated at 4 to 6 months and 8 to 12 months.
- Thereafter, cattle should be vaccinated at routine postcalving examinations.

Vaccination of bulls is of questionable value because they will be seropositive on blood testing and may be rejected for sale as being infected. Routine examination of semen for the presence of the virus is preferable as a method of control.

Bovine Herpesvirus 4

Bovine herpesvirus 4 (BoHV-4) is present worldwide and can affect cattle and other domestic and wild ruminants. The virus usually results in a subclinical infection, but it can cause reproductive disease such as endometritis, vulvovaginitis, and abortion. The virus was first isolated from a case of endometritis by Park and Kendrick (1973) and has subsequently been found in cases of endometritis and metritis in several countries. It has a specific trophism for endometrial stromal and epithelial cells (Donofrio et al. 2007), which suggests that its role may be as a primary pathogen rather than merely a coincidental isolate. Likewise, Czaplicki and Thiry (1998) have associated seropositivity to BoHV-4 with abortion, and infection of the fetus by the virus has also been demonstrated (Kendrick et al. 1976).

The BoHV-4 virus can be detected by PCR, but because of the carrier status that occurs with herpesviruses, the presence of the virus does not necessarily equate with its causal role in disease. Serology, using an ELISA, is also possible (Anon 2017).

Bluetongue

Bluetongue is an infectious, non-contagious, vector-borne disease that affects domestic and wild ruminants. It is mainly a disease of sheep and deer, but cattle and wild ruminants are important reservoir hosts for the virus. Cattle are particularly significant in the epidemiology of the disease due to the prolonged viraemia in the absence of clinical disease (OIE 2017f). Bluetongue is found mainly in countries between 40°N and 35°S (Radostits et al. 2007) and is endemic in the western states of the US. Since 1999, there have been significant outbreaks of the disease in Europe. Initially, they were in southern European countries (Greece, Italy, Corsica, and the Balkans), but since 2007, there have also been outbreaks in Germany, France, the Low Countries, and the UK (European Union Reference Laboratory, 2017). Bluetongue is not present in Canada and New Zealand. In Australia there is evidence of its presence, but there is no clinical evidence of disease (Kirkland 2004). There are at least 26 serotypes of bluetongue (Maan et al. 2012) that are classified antigenically and taxonomically as bluetongue virus, but each serotype is unique and may not cause clinical bluetongue disease (Walton 2004). Different serotypes have different geographical distributions.

The virus requires insect vectors for its transmission and is not normally transmissible from animal to animal. *Culicoides* midges are the main vectors in the US, where the main agent is *ulicoides sonorensis*. In Africa and southern Europe, the main aent is *C. imicola*. The recent spread into areas of central and northern Europe that are beyond the northern limit of occurrence of *C. imicola* suggests that new insect vectors are involved: namely, *C. obsoletus* and/or *C. pulicaris,* which are widely distributed throughout northern Europe, appear to be the most likely candidates (Mertens & Mellor 2003). Different serotypes are associated with different primary vectors (Walton 2004). Competent vectors for bluetongue are present in Australia, but the geographical separation between vector-prone regions, and cattle- and sheep-raising areas would mitigate the risk of an incursion of bluetongue virus obtaining access to the national sheep flock (Tay et al. 2016).

Outbreaks of bluetongue have generally been described as being worse in the summer than in the winter, according to the number of vectors in the environment. Brand and Keeling (2017) have suggested that periods of warmth, in which insect biting is maximal, followed by cooler weather to prolong the life of the insect, may be associated with high intensity of bluetongue transmission, and that epizootics (at least in more marginal ranges for the vectors such as the UK) are associated with the prevalence of such weather patterns. It appears that the outbreak in northern Europe has also been accompanied by a change in the infectivity of the virus, such that it can persistently infect ovine T cells, thereby having a more effective mechanism for 'overwintering' between one vector season and the next (Mertens & Mellor 2003). There may also be some possibility of transmission by ticks, keds, and mosquitoes (Radostits et al. 2007). Bulls that are infected by bluetongue virus can transmit the virus in their semen (Bowen & Howard 1984), and it is possible for the virus to be transmitted by direct transfer of blood from an infected animal.

In cattle, clinical disease is rarely caused by bluetongue virus (Radostits et al. 2007), but it does have a number of effects upon bovine reproduction. Several serotypes have been identified as causing fetal infection (Savini et al. 2012) and, during the European epizootic outbreak in 2007 to 2008, serotype 8 (BTV-8) was recognised to have the ability to cross the placenta and result in fetal loss and the birth of viraemic offspring. Infection before Day 100 of gestation can lead to abortion, mummification of the fetus, or stillbirths. Nusinovici et al. (2012) found that exposure of naïve herds to BTV-8 virus was associated with an increase in the occurrence of abortions, regardless of the stage of pregnancy. Overall, there was a 7% increase in late returns to service, but exposure in the first 3 months of pregnancy was associated with

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a 15% increase of late return-to-service. Calves may be born alive that are weak and ataxic or are persistent carriers of the infection (Roberts 1986). However, teratogenic effects are more likely than abortion (Kelling 2007). The neuropathogenicity of the virus produces hydranencephaly (Howard 1986) and cerebral cysts, sometimes with consequential abnormal contractures of extremities. Live-born calves can show clinical signs of abnormal behaviour ('dummy calves') such as circling, head pressing, incoordination, and blindness (Wouda et al. 2009); the later in gestation the infection occurs, the more mild the effect upon the fetus is likely to be.

In the aborted fetus, diagnosis of bluetongue can be made by demonstration of central nervous lesions (Barr & Anderson 1993) or by virus isolation from fetal blood, spleen, lung, or brain. PCR is now the preferred method of identifying the virus, with serogroup-specific tests or gene-sequencing used to identify the virus serotype (OIE 2017f). Serology (serum neutralising assay or ELISA) can be used to diagnose maternal infection, although the presence of antibody–negative, viraemic animals during an epizootic outbreak can confuse diagnosis (Osburn et al. 1981).

Live attenuated vaccines are available for bluetongue, which generally confer good protection against infection and abortion. There is evidence that serotypes 2 and 9 in attenuated live vaccines have caused abortions in their own right (Savini et al. 2012), but the proportion of abortions (i.e., compared with total vaccinated animals) was very low, and the recovery of vaccinal strains from abortuses lower still. There is also a risk of attenuated vaccine strains reverting sufficiently to 'wild type' that they can be transmitted through vectors (Savini et al. 2008). Hence Osburn (1994) recommended that, if vaccination is used, it should be confined to the vector season. Vaccines are currently under development that utilise purified viral proteins rather than attenuated live strains.

Schmallenberg Virus

Schmallenberg virus is a member of the family Bunyaviridae and a member of the Simbu serogroup, together with Akabane virus (Wernike et al. 2015). Schmallenberg virus was first reported in 2011 as an unknown disease of dairy cows associated with non-specific signs of fever, diarrhoea, and decreased milk production. Subsequently, calves were born that showed different degrees of malformations. After ruling out other known pathogens, the virus was isolated and named the Schmallenberg virus. The virus affects wild and domestic ruminants and has also been detected by serology in wild boar and alpacas. It is spread by biting *Cullicoides* midges (C. *obsoletus, C. dewulfi, C. chiopterus, C. scoticus, C. punctatus,* and *C nubeculosus*) (Kauffold et al. 2014b). It spread rapidly through Europe in the years after 2011, is also present in Turkey, and may be present in Jordan. It has not yet been reported from elsewhere in the world (OIE 2017g). However, Sohier et al. (2017) noted an increase in cases in Belgium in 2016 and suspect that the virus may develop a 4- to 6-year cycle of activity. The prevalence of the virus in herds (> 95%) and of animals within a herd (> 85%) is high (Veldhuis et al. 2013).

In adult cows, the signs include fever, reduced milk production, and diarrhoea. In naturally infected pregnant cattle, vertical transmission of the virus to the fetus can occur between Days 80 and 150 of pregnancy (Kirkland et al. 1988), resulting in embryonic mortality, fetal malformations, abortions, and stillbirths in approximately 3% of calves (Dominguez et al. 2014). Birth defects include arthrogryposis, scoliosis, sunken eyes, cataracts, maxillary retraction dental irregularities, hydranencephaly, and cerebellar hypoplasia. Limb contractures or deformities can occur, which can also result in dystocia. Most affected calves are born dead (Kauffold et al. 2014b).

The presence of Schmallenberg virus can be demonstrated by PCR or by virus isolation from cows during the period of viremia. In stillborn or malformed newborns or abortuses, the virus can be similarly demonstrated in fetal brain, spleen, meconium, and amniotic fluid (Bilk et al. 2012). Once cattle become infected with Schmallenberg virus, they develop long lasting immunity (Méroc et al. 2015), and virus specific antibodies in the dam can be detected by virus neutralisation, indirect immunofluorescence, or ELISA (Kauffold et al. 2014b). Bulk milk antibody ELISA can also be used as a means of monitoring the presence of the infection in a herd; it is generally highly correlated seroprevalence among cows (Collins et al. 2017), although there have been occasions when bulk milk has tested positive despite only a few seropositive animals in a herd (Daly et al. 2015).

There is no specific treatment against the Schmallenberg virus. There are two inactivated-virus vaccines available in the UK and France, which can be used in sheep and cattle. Cattle require two vaccinations are required at an age ≥2 months and again 3 weeks later. It is not yet known how long protection may last, but is assumed to be ≥12 months. Additional control may be through control of the vector or ensuring that animals are at the susceptible stage of pregnancy during the vector-free season (Kauffold et al. 2014b).

Akabane Virus

Akabane virus was first isolated in Japan in 1959. It is classified in the Simbu group of the Bunyaviridae family along with Schmallenberg virus, with which it shares many similarities. Akabane virus has been reported in a number of countries in the African continent, the Middle East, Southeast Asia, and Australia. It is also transmitted by *Culicoides* midges, and it is likely that the vectors of bluetongue are also effective as vectors for Akabane virus (Kirkland 2015). There is a seasonal pattern to the disease. In temperate countries, the vector in most abundant in the summer, so the disease follows suit. In warmer countries, the disease is less common in the dry season – again, in parallel to the abundance of the vector. Changing or unusual climatic conditions allow the vectors to spread beyond their usual range, where they encounter naïve cattle that are susceptible to infection (Tranter et al. 2010).

In a naïve animal a bite from an infected midge results in multiplication of the virus at the site, followed by viraemia within 1 to 6 days. In most cases, infection produces no overt clinical signs, although some strains of Akabane virus can cause encephalitis in adult cows or in newborn calves.

In naïve pregnant animals, the virus can spread to the placenta where it replicates and spreads to the fetus. The virus then multiplies in cells of the fetal brain and spinal cord, resulting in hydranencephaly and/or arthrogryposis (Parsonson & McPhee 1985). There is no evidence that the virus can affect the early embryo before the development of the placentome, so most of the damage is seen in calves whose dams were infected between 3 and 6 months of gestation. The first sign of the infection in a herd is abortions at 4 to 6 months of gestation; some limb deformities may be present, but severe hydrancephaly will be revealed at post mortem examination (Kirkland 2015). Calves that are infected at up to Day 120 of gestation are likely to show hydrancephaly, and those infected between Days 105 and 150 of gestation are likely to show arthogryposis of multiple joints and limbs. Calves infected later in gestation may show signs of acute encephalitis, such as flaccid paralysis of the legs, hyperextension of joints, and difficulty

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in standing. Once the fetus has become immunocompetent, damage is less apparent or does not occur (Tranter et al. 2010). Thus the incidence of defects in calves may be as high as 50% if cows are infected in the third or fourth month of gestation, declining to approximately 25% in the fifth month and approximately 5% in the seventh month (Kirkland et al. 1988).

Serology (ELISA or virus neutralisation) of the fetus or neonate is the most useful diagnostic test. The presence of antibodies to Akabane virus in precolostral serum or fetal fluids is strong evidence that the calf was infected in utero. However, the absence of specific antibodies does not exclude the diagnosis if infection precedes the development of immunological competence. Maternal serology is only of value if the affected cattle are from a region where the virus is not endemic (Tranter et al. 2010). PCR may be considered to detect virus in tissue of an aborted fetus or in cotyledons of the placenta; various refinements of the basic method have been published to improve the sensitivity of the diagnosis (Shirafuji et al. 2015) or to rapidly differentiate between Akabane and Schmallenberg virus (Lee et al. 2015).

The effects of Akabane can be controlled by vaccination of susceptible animals before exposure to vectors. Various live and inactivated vaccines are currently licenced for use in Japan and South Korea (Iowa State University 2017). Inactivated vaccines have the advantage of being suitable for the emergency vaccination of pregnant animals. Vector control may be considered as may the feasibility of avoiding having animals at the susceptible stages of pregnancy during the vector season.

Epivag

Epivag is a specific bovine venereal disease causing epididymitis and vaginitis in cattle in east, south, and central Africa (Hudson 1949, Roberts 1986). In cows, it causes diffuse infection of the vagina, characterised by a reddened mucosa, but without ulcers, erosions, or vesicular lesions. A severe mucopurulent vaginal discharge may be present during the earlier stages of the disease. Most infected cows fail to conceive to service. Many eventually recover, but about 15% to 25% of animals become sterile because of the presence of lesions of the uterine tubes such as adhesions, hydrosalpinx, and ovarian and bursal adhesions. Likewise, some cows develop parametritis as a result of epivag infection (McEntee 1990), and adhesions may be widespread throughout the pelvis and even extend into the abdomen.

Most bulls have a mild balanoposthitis after infection, although, because this is far less severe than IPV infection, it may not be observed. Subsequently, most bulls develop an induration of the epididymis, particularly of the tail. Testicular degeneration, atrophy, and fibrosis are a common sequent of the infection, and orchitis may also occur (Rocha et al. 1986).

The causal organism has not been definitively characterised. Theodoridis (1978) partially characterised a series of viruses from cattle with the epivag syndrome, including some that were related to bovine herpesviruses. However, although the vaginitis component of the syndrome could be induced by various strains of these herpesviruses, the epididymitis could not. Hence it remains unclear whether the syndrome is caused by a bovine herpesvirus and, indeed, whether herpesviruses are the sole causal agent.

Catarrhal Bovine Vaginitis

This contagious, mainly venereally-transmitted, disease was first described in South Africa (Van Rensburg 1953); since then it has been reported in many countries. It is believed to be caused by an enterovirus from the enteric cytopathic bovine orphan (ECBO) group (Straub & Böhm 1964), although this remains to be proven. Transmission of the disease is primarily by the venereal route, but it can also be spread by faecal contamination of the vulva or by animals licking the perineum of infected and non-infected individuals. Therefore the disease occurs in virgin heifers.

Affected cows have a profuse, postcoital, non-odourous, yellow, mucoid vulval discharge. The cervix and vagina are inflamed, but there are no pustules such as occur in IPV infection and no fever. The typical yellow gelatinous exudate frequently accumulates in the vagina, varying in quantity from a few to several hundred millilitres. The disease persists for a few days to a few weeks. Only a few animals show clinical signs of the disease at any one time. As a consequence, pregnancy rates are reduced, and there are prolonged, irregular returns to oestrus, presumably due to late embryonic death. In some herds, fetal mummification, abortion, and stillbirth have been reported as being a problem. Bulls may or may not become clinically infected, but ECBO serotypes have been associated with seminal vesiculitis and infertility lasting up to 90 days (Bouters et al. 1964).

Diagnosis can be made on serological examination of paired blood samples collected at least 15 days apart for evidence of rising antibody titres; the first sample should be collected as soon as possible after the disease is suspected. The virus can be isolated from vaginal mucus, but the recovery rate is frequently low (Huck & Lamont 1979).

There is no specific treatment or vaccine. Infected bulls should not be used for service for several months even after clinical signs of disease have disappeared. Potentially infected animals should be isolated after purchase, and in closed herds serological examination of potential additions to the herd might be contemplated.

Transmissible Genital Fibropapillomas

Wart-like tumours commonly occur on the penis of young bulls (see Chapter 36), and occasionally, similar growths occur on the vulva, perineum, and vestibulovaginal epithelium of heifers. They are caused by a virus of the papovavirus group and are transmitted by contact with infected animals.

These fibropapillomata typically regress spontaneously in 2 to 6 months; the speed of regression may be expedited by the use of a wart vaccine (formalised tissue). Except insofar as the larger tumours (which may be removed surgically) might interfere mechanically with coitus, they do not cause infertility in female animals.

Fungal Agents (Mycotic Abortion)

Fungal invasion of the placenta and fetus is a frequent and consistent cause of abortion in cattle (Table 24.1). Abortions are normally sporadic, although in some herds the incidence may be as high as 5% to 10%. The frequency of diagnosis is high in the northeastern states of the US, in which mycotic abortions accounted for 22% of all infectious abortions and 5.1% of all abortions investigated (Hubbert et al. 1973). Similarly, in South Dakota, US, a survey over a 5-year period found that 14.6% of all infectious abortions were due to fungi; this was 4.8% of the total number of abortions (Kirkbride et al. 1973).

Aetiology and Pathogenesis

The fungi that are most frequently isolated after abortion are *Absidia* spp., *Rhizopus* spp., *Mucor* spp., and *Aspergillus* spp. Other fungi

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such as *Mortierella wolfii* and *Petriellidium boydii,* together with yeasts such as *Candida* spp., have also been implicated. Worldwide, *Aspergillus fumigatus* is the most common cause of abortion (Pepin 1983), being the cause of 60% to 80% of mycotic abortions (Knudtson & Kirkbride 1992), but other species can be locally important. In the North Island of New Zealand, for example, *M. wolfii* is the most important causal organism.

In the UK, mycotic abortion is much more prevalent during the months of December to March than during the rest of the year. Abortions are associated with the feeding of poor quality hay and silage, particularly when cows are also in confined housing (Williams et al. 1977). It is assumed that infection of the placenta and fetus occurs as a result of haematogenous spread of the organism from the alimentary or respiratory tract. Infection progressively spreads through the placentomes, causing abortion once too much of the placenta has been affected for the fetus to remain viable. Evidence of infection in the fetus is frequently also present, particularly the skin and lungs (Walker 2007). Mycotic infection does not invariably cause fetal death and abortion; sometimes, infected calves are born alive.

Clinical Signs

Abortions usually occur between the fourth and ninth months of gestation, with most occurring between the seventh and eighth months. When present, the appearance of lesions on the fetus and placenta are characteristic of mycotic infections. The whole or part of the placenta appears discoloured grey, yellow, or reddish-brown, with the intercotyledonary areas of the allantochorion appearing thickened, wrinkled, or leathery. Those cotyledons that have attached portions of the corresponding caruncle after the placenta has been shed appear thickened and have a cup-like or coffee bean appearance (Pepin 1983). Between 25% and 33% of fetuses have characteristic skin lesions (Austwick 1968, Kendrick 1975), which are circumscribed, greyish-white thickened patches similar in appearance to skin ringworm in calves and young cattle. These appear dry when *A. fumigatus* has caused the abortion and moist when it is due to *Zygomycetes* (Walker 2007). Skin lesions are not normally present in *M. wolfii* abortions.

There are no other clinical signs of disease in the dam associated with abortion due to *A. fumigatus*. A significant proportion of cows that abort because of *M. wolfii* infection develop mycotic pneumonia that is invariably fatal a few days after abortion.

Diagnosis

The presence of typical lesions of the placenta and fetus is highly suggestive of mycotic abortion. The main differential diagnosis is *Bacillus licheniformis*.

Laboratory confirmation requires submission of placental tissue, preferably the whole organ (Pepin 1983). Culture from placental tissue is of no value because the placenta is usually contaminated after it has been expelled. Culture from fetal lungs and abomasum is more reliable, but contamination can occur.

Mycotic abortion can be diagnosed by demonstrating fungal hyphae in the placenta, either histologically or in scrapings after digestion in 10% KOH solution (Pepin 1983). Fixed placenta is the best sample to submit. A range of other tissues (including lung, liver, and brain) should be examined histologically if placenta is not available. Fetal bronchopneumonia is common with all types of mycotic abortion and, if present, is considered diagnostic.

Conclusive diagnosis of mycotic placentitis can be made if (Kirkbride 1990):

- Characteristic lesions of placentitis are present in association with the presence of mycotic elements.
- Characteristic lesions of fetal dermatomycosis are present in association with the presence of mycotic elements.
- There is a fetal bronchopneumonia associated with mycotic elements.

Serological tests are, at present, unreliable and cannot be used for routine diagnosis.

Control

There is no practical treatment for mycotic abortion. The feeding of mouldy forage or the use of mouldy bedding should be avoided. There is no point in examining the suspect feed because *Aspergillus* and other fungi are normal inhabitants of such forages and will almost invariably be present.

Diseases of Unknown Aetiology

Foothill Abortion (Syn. Bovine Epizootic Abortion)

This disease was first identified in the mid-1950s in California. It is characterised by a high, late term abortion rate (30%–40%) during the last trimester of gestation or by the birth of weak calves to cows and heifers newly introduced to beef herds in particular areas of the states of California, Oregon, and Nevada (Barr & Anderson 1993). Abortions are confined to the habitat of the argasid tick *Ornithodoros coriaceus,* which appears to be an essential vector for the disease.

Identification of the causal organism was difficult. Early studies suggested that the disease was due to *Chlamydia* spp. However, it progressively became evident that this is not the case, and that foothill abortion is a separate disease entity from bovine chlamydial abortion (Barr & Anderson 1993). The early literature remains confusing as a result. Recent studies using PCR on aborted fetuses and the tick vector (King et al. 2005, Chen et al. 2007) have identified a novel deltaproteobacterium, which is closely related to *Polyangium cellulosum,* a member of the order Myxococcales, as the aetiological agent (Stott et al. 2015).

Abortions are seasonal, occurring 3 to 4 months or more after exposure to ticks. The agent appears to infect the fetus by crossing the placenta from the infected dam. Infection of the fetus needs to occur at about 60 to 140 days of gestation for fetal disease to occur. Most abortions occur in the third trimester, usually as sporadic occurrences but occasionally as a 'storm'. Cattle that abort show no other clinical signs, either at the time of abortion or subsequently. Once abortions have occurred, animals are immune, so the cattle that are at greatest risk are those calving for the first time and animals that have been moved into a tick-infested region (BonDurant et al. 2007). Infection late in pregnancy can give rise to the birth of live, weak calves (Barr & Anderson 1993). It is not yet known whether infection early in pregnant can result in early embryonic death.

Lesions in aborted fetuses are characteristic and are used in its diagnosis. Abortuses are typically not autolysed but have enlarged lymph nodes, spleen, and liver and a reduction in the size of the thymus (Jubb et al. 1993). There may be petechial haemorrhages of the mouth, tongue, thymus, lymph nodes, and elsewhere (Storz 1971). Histology is required to confirm the diagnosis; there is initial lymphoid hyperplasia, which may be followed by acute necrosis of lymphoid organs (BonDurant et al. 2007). Although the causative organism has not been definitively identified, its

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presence in abortus material can be identified by a PCR against the 16S bacterial ribosomal gene (Stott et al. 2015).

Control is attempted by ensuring that susceptible animals are exposed to ticks before they become pregnant. Although simple in theory, it is difficult in practice to find a 'window' of time between the periods of risk from ticks and the cattle being in sufficiently advanced gestation to avoid abortions.

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Veterinary Control of Herd Fertility in Intensively Managed Dairy Herds

CHRIS HUDSON, JOHN GEORGE COOK AND RICHARD LAVEN

Introduction

Background

Control of cow fertility is critical to running a profitable dairy enterprise because of the shape of the cow's lactation curve. Daily milk production increases after parturition up to a peak (usually at 40–80 days postcalving in mature cows), after which it declines with time. This effect is much less marked in first lactation heifers, which typically have much 'flatter' lactation curves, but it is still important. Therefore in order to produce milk efficiently, cows are required to calve at regular intervals throughout their productive lives so that they spend most of their time in the relatively productive part of the lactation curve.

Both artificial insemination and natural service are common in the main dairying nations, and many herds use a combination of both. Cows are commonly considered eligible for breeding after a set period of time after calving (the voluntary wait period, VWP) in herds calving year-round and during a specified window of time during the year in seasonal calving herds. The choice of VWP policy is determined by the herd manager and is usually influenced by lactation curve shape and herd replacement policy. Cows that fail to become pregnant sufficiently quickly after becoming eligible for breeding are commonly culled from the herd; the decision on the stage at which this happens typically varies between cows.

Evolution of Dairying and Fertility Management

Dramatic changes have occurred to the global dairy industry over recent decades, brought about through improvements to management, feeding, and breeding practices. Genetic advances have led to breeds that are heavier, leaner, and have a higher milk yield potential. Deregulation of supply in many markets has also exposed the dairy producer to the effects of widely fluctuating milk prices, as dairy commodity prices rise and fall in response to global influences as well as local circumstances.

Although the future is difficult to predict, it seems likely that there will be a continued general increase in herd size, alongside an increase in the number of farms producing milk aimed at small, but high value, markets. Large herd size offers the potential to reduce overhead costs per litre in exchange for a greater market share, whereas the niche market benefits from a potential higher product value. The dynamics of health and fertility management are fundamentally different in larger herds, and the challenge for herd owners and veterinary surgeons is to understand how their roles need to change. The obvious and clear incentive that should motivate dairy producers to improve health and fertility is the undoubted financial benefits that can be gained from such improvements, and in larger herds it is logical that these improvements should be carried out in an evidence-based and planned manner. However, in many cases, early 'health planning' initiatives simply led to the construction of documents solely designed to comply with the requirements of farm assurance schemes and large retailers. A clear distinction should be drawn between this approach and a more holistic approach to herd health and production monitoring, which should be a continuous, active, and ongoing process involving regular monitoring of key parameters and processes; this requires a detailed knowledge of the farm as well as excellent record keeping.

Arguably, there is no reason why a similar approach should not be undertaken for herds of all sizes. Nevertheless, although both large and small dairy herds face the same reproductive management issues, in larger herds the problems become magnified, and the potential economic consequences are larger and accrue faster. Larger herds are characterised by some fundamental differences that go beyond their size and structure, which necessitate a different approach to the problem of reproduction.

The provision of labour, both in terms of quantity and quality, is a key area as herds expand in size. Larger herds involve more cows and more staff, but generally fewer people per cow; many large herds operate at around 1 person per 120 cows. Historically, dairy herds have relied on skilled individual staff to retain a high level of individual cow knowledge, but as the herd gets bigger, this level of individual attention to detail becomes impossible to sustain. At a certain herd size, it becomes necessary to replace this day-to-day individual cow knowledge (what is essentially a data capture and storage function) with technology, usually in the form of computer software packages. Technology means that the skill levels of the average farm worker do not need to be as high as in smaller herds, but key skills areas remain that, as yet, cannot be successfully replaced by technology, such as evaluation of calving issues or the carrying out of artificial insemination (AI) procedures. When technology can have an effect, it should go without saying that there should be a cost benefit. For example, the use of pedometers to aid oestrous detection may improve submission rates but potentially could incur increased costs if logistic considerations, such as cow identification and handling and separation facilities,

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are suboptimal; as a consequence, greater labour costs may be incurred in physically finding the cows to serve.

Increased numbers of staff mean that senior staff must learn to interact and manage subordinate personnel, and there is no doubt that this represents a huge challenge on larger units. Personnel management (and sometimes language) skills are needed that are not necessary in a smaller herd employing maybe a single member of staff. Greater numbers of employees incur a greater staff turnover, and issues with sickness, holiday, and work rotas can be disheartening; although the overall skill level may be reduced, herd performance should not be limited by the least skilled worker. As herds expand, job roles, such as milking, may become more mundane and routine, whereas other roles become more specialised such as, for example, that of parlour manager. Systems will need to be in place to ensure that all staff are adequately trained to allow them to carry out their job role to a given standard and that performance is monitored. Motivation of staff, continuing education, and the opportunity for progression may be crucial for success in many larger herds. The importance of high quality data and data management cannot be overemphasised in larger units. However, as time spent at the computer keyboard increases, it becomes vital that methods of data capture are as automated and speedy as possible, so the time that key managers spend in the cow-centric actions resulting from that data increases, and less time is spent inputting it.

Cows in larger herds are often split into multiple management groups, but group sizes of over 200 are common, resulting in more cow-to-cow interactions. However, the effects of behaviour and group sizes on health, welfare, and production are not well understood and will vary depending on herd facilities. Larger herds can consider management strategies, such as milking heifer-only groups, to mitigate some of these effects. Larger groups theoretically may improve oestrous detection, as more cows will be simultaneously sexually active. In reality, differentiating and identifying cows with short duration and less intense oestrous expression can be very difficult in large groups.

For the herd owner, changing to an environment with more staff to manage, a reliance on data, and a magnification of cost structures requires considerable adaptation and learning. Senior managers in large herds often have high levels of formal education and an in-depth knowledge of both the biology of the dairy cow and business management; this drastically changes the level and quality of input required from their professional advisors.

Veterinary surgeons working with large herds should realise that there is a shift in emphasis towards the use of data and team approaches and a clear focus on profitability. Critical to the management of larger herds is to have systems in place that monitor critical indicators and provide an early warning system of health and production problems. Many of the traditional roles and technical tasks carried out by veterinary surgeons in smaller herds will often be undertaken by trained herd staff; the role of the veterinary surgeon is to provide an independent evaluation of those systems and any changes proposed.

As the dairy industry has evolved, the techniques used to manage reproduction have also evolved. Between 1956 and 1967, research took place that largely established the mechanism of luteolysis (see Chapter 1). In 1979 the first prostaglandin (PG) product (Lutalyse, dinoprost trimethamine) was licensed for use in cattle by the United States Department of Agriculture (USDA), becoming the first pharmacological agent used to manipulate reproduction in dairy cows. The first systematic and structured use of these products was termed 'targeted breeding', in which cows were injected on either a weekly or a fortnightly basis, and then either inseminated at observed oestrus or inseminated at a fixed time interval after the injections were administered. The procedure could be further refined by transrectal veterinary palpation or, later, ultrasound examination of cows to identify those animals likely to have a corpus luteum (CL) and therefore thought more likely to respond to treatment.

Conception rates from insemination to observed oestrus occurring after prostaglandin administration proved to be acceptable. In contrast, the results of inseminations conducted at a fixed time were disappointing because the timing of ovulation relative to prostaglandin injection was highly variable depending on the stage of follicular wave development at the time of the prostaglandin injection.

This led to the development of the 'Ovsynch' protocol, which has now become widely adopted globally within the dairy industry as a breeding tool to ensure timely insemination of cows (Pursley et al. 1995; see Chapter 8 for further details). This protocol offered dairy producers the first opportunity to adopt fixed time insemination as a viable management strategy. More recently, it has been shown that conception rates could be improved if the 'Ovsynch' protocol could be commenced during a particular part of the cycle (day 4–12); this led to the development of presynchronisation protocols (see Chapter 8 for further details).

In some large herds, especially in North America, it has become common practice to breed all cows by timed AI using synchronisation programmes. For example, Souza et al. (2013), based on a survey of over 1 million inseminations from 40 US states, reported that 30% of all inseminations were timed AI after a synchronisation programme. This is for a variety of reasons. First, large herds may find it more convenient and cost effective to organise labour and work routines in a consistent manner so that certain tasks always occur on a certain day of the week. This can allow skilled staff to focus more on certain other aspects of cow care at specific times and, by doing so, generate other health and welfare benefits. Second, in some herds, suboptimal oestrous detection prevents generation of sufficient pregnancies to sustain viable production, leading to adoption of 100% timed AI.

However, the routine use of synchronisation programmes, without any attempt being made to identify natural oestrus (which is particularly common when presynchronisation programs are used), combined with conception rates that are usually around 35% (Souza et al. 2013), means that on many farms a high proportion of cows are receiving 10 or more hormone injections or treatments before they become pregnant and some are receiving more than 20. This is undoubtedly an ethical and an animal welfare issue, especially when routine synchronisation is used without addressing the underlying issues causing the need for that synchronisation. This is consistent with the findings of Higgins et al. (2013), who reported that 65 of 93 UK dairy veterinarians judged the long-term routine use of synchronisation programmes as "unacceptable" if they were used at the start of the breeding period without addressing the underlying issues causing the fertility problem. This begs the question that if one of the key underlying causes of the poor fertility in the herd is its genetic make-up, does continuing to breed for production, without a focus on fertility, count as a failure to address the underlying causes?

The majority of respondents (> 70%) to Higgins et al. (2013) wanted to see a decrease in the use of hormones to control fertility in dairy cattle. Achieving such a decrease may be difficult. First, there was a considerable variation in attitude across the respondents in the study by Higgins et al. (2013), and it is highly likely that veterinary perspectives on the routine use of hormones may differ across different systems (for example, in North America it is likely that acceptance of routine treatment would be higher

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TABLE 25.1 Examples of published levels of reproductive performance worldwide

Nation	Year	Parameter	Reported Average	Trend Over Time	Reference
US	2006	CI CR	422d 30%	Deterioration in both measures 1996–2006 (CI more marked)	Norman et al. (2009)
Canada	2011	21d PR SR CR	14% 38% 37%	Relatively stable for each measure 1999–2011	Le Blanc (2013)
UK	2015	CI CFS CR	410d 80d 32%	Improvement in most measures 2010–2015, CR stable	Hanks & Kossaibati (2016)
Norway	2006	CI CFS	378d 86d	CI relatively stable 1989–2006	Refsdal (2007)
Sweden	2005	CI CFS	403d 92d	Deterioration when 1995 compared with 2005	Löf et al. (2007)
Ireland	2000	CI	395d	Deterioration 1991–2000	Mee (2004)
Australia	2009	6 wk ICR 3 wk SR CR	50% 72% 38%	Slight deterioration in 6 wk ICR 2000–2009 (others not reported)	Morton (2011)
New Zealand	2010	6 wk ICR 3 wk SR CR	67% 81% 48%	Not reported	Brownlie et al. (2014)

CI, calving interval; *CR*, conception rate/risk; *21d PR*, 21-day pregnancy rate/risk; *SR*, submission/serve rate; *CFS*, calving to first service interval; *6 wk ICR*, 6-week in calf rate; *3 wk SR*, submission rate in first 3 weeks of breeding period.

than in the UK, where fixed time AI is used alongside oestrous detection). Second, hormone treatments are a significant source of income for veterinary practices, and it is likely that a focus on management rather than treatment will reduce veterinary incomes (Higgins et al. 2013). For these reasons, any decrease in the use of hormones for routine oestrous synchronisation is likely to come from pressure from outside the industry (as happened in New Zealand when the use of corticosteroids for inductions at the end of pregnancy was banned).

In the authors' opinion, there should be few situations in which the reasons driving the use of synchronisation programmes cannot be addressed and performance improved without necessarily using hormones. Combining good oestrous detection with appropriate synchronisation of target cows provides a comprehensive system of sequential 'pregnancy traps' as cows progress through lactation.

Using this reasoning, many large herds find that sufficiently high levels of reproductive performance can be achieved and sustained by applying protocols at the herd level and thus removing the need to individually examine each cow before deciding on an appropriate course of action. Substantial cost savings can also be made by abandoning individual cow level decisions. In these situations, it is vital that veterinary surgeons have a thorough understanding of herd level risk factors if their actions and decisions are to remain relevant and add value to a dairy business.

Levels of Reproductive Performance and Trends over Time

Typical levels of reproductive performance vary widely across the dairying nations of the world, with poorer overall performance usually encountered in systems involving high milk yields and better performance often associated with seasonal calving. Examples of publications describing reproductive performance measurements from samples of herds worldwide are summarised in Table 25.1.

Across most areas, there is a medium to long term declining trend in fertility (Brownlie et al. 2014, Hudson et al. 2010, Morton 2011). This is commonly associated with rapidly increasing milk production, which not only may compromise fertility by making cow energy balance more challenging to manage but also as a consequence of genetic selection focused heavily on production merit (Lucy 2001, Royal et al. 2000). However, during the last decade the trend has halted or even reversed in many countries; this may be associated with an increased understanding of the economic importance of fertility, adoption of new technology to assist breeding, and the more widespread use of fertility measurements as part of genetic selection indices.

Evaluation of Herd Fertility

Herd Recording Systems

Accurate recording of reproductive events is critical, both for individual cow management decisions and to allow effective monitoring of herd reproductive performance. Irrespective of how events are recorded, the approach should be consistent (e.g., between members of staff and over time) and comprehensive. The events that should be recorded are outlined in Table 25.2, including an indication of the minimum level of data recording required for useful analysis, as well as additional information that allows greater insight if collected.

In addition to these events, some metrics and monitoring approaches also require evaluation of 'metadata' for the herd – for example, the herd's VWP policy or the date of the planned

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TABLE 25.2 Reproductive events which should be recorded in a dairy herd to facilitate effective monitoring

Event	Minimum Information	Additional Information
Calving	Cow ID, date, parity	Level of intervention, calf outcome
Insemination	Cow ID, date	AI/natural, sire, operator (for AI), observed heat/FTAI
Pregnancy diagnosis	Cow ID, date, result	
Reproductive disease		Cow ID, date
Oestrus no serve		Cow ID, date
Other veterinary examination		Cow ID, date, result, treatment

start of mating. Depending on the computer software being used for analysis, the user can often add these additional parameters.

A wide variety of different dairy herd management software systems exist that can be used to record and store these data, and this varies widely in different areas of the world. In some cases the herd management system is linked with the milking plant, but specialised stand-alone software is also very common. For most medium to large dairy enterprises, efficient management is very difficult without the use of on-farm computerised records. A major challenge in analysing fertility performance of a herd is the variation in the degree to which the various software systems are able to calculate and present reproductive performance measurements. Commonly, different programmes calculate different metrics and often use different calculations or methods so that different values for the same metric can be derived from the same set of data, depending on which software is used. One approach to this problem is the development of analysis software, which can use data from varied sources and apply analysis in a consistent fashion.

Bureau recording systems (in which a third-party organisation collects and stores farm data) represent an alternative or supplement to on-farm software systems. Commonly, this service is provided by organisations also supplying milk quality testing, such as milk recording organisations or dairy herd improvement associations. These systems can usually accept data input either from on-farm paper records or by extracting data from an on-farm software system. Some bureau recording systems also provide an Internet-based portal where farmers can enter event data directly into the central database. Analysis of this collated data is then commonly available via a dedicated website; the degree of reproductive monitoring available through such systems varies greatly between providers and is often more limited than would be accomplished using specialised monitoring software. In addition, some bureau services allow downloading of their data for use by farmers and/or advisors; this can provide a way to obtain data in a consistent format for analysis, but sometimes data quality is reduced or event detail lost from the original source.

Irrespective of the source of the data, it is important to treat it critically and to recognise that poor data quality can influence the results of analysis. The most common problem is missing event records. For example, when not all inseminations are recorded (the final, successful insemination of the lactation being the most likely to be captured), the herd's performance in terms of submission rates for service will appear artificially poor, whereas the conception rate will be artificially inflated. There are algorithmic approaches to evaluating fertility data quality, and some software products offer data quality assessment.

There is a clear trend throughout the agricultural industry for increased amounts of data to be generated and recorded over time. Examples from the dairy sector would include the more widespread adoption of sensor technology such as activity monitoring as a heat detection aid. The ability to integrate these data with that from other sources and use it to generate additional insight is likely to become ever more important to the veterinary advisor in the future.

Aspects of Reproduction which Should Be Monitored

Herd-level reproductive performance can be monitored in a variety of ways, but whichever measures are used, it is important that they cover the relevant areas comprehensively. A hierarchical approach can be useful, in which a small number of measurements of overall reproductive performance are used as the main short-term metrics and a wider set of measurements covering the components of reproductive success used when the headline performance is suboptimal. The two key elements that should be covered are submission (the ability of cows to express oestrous behaviour and the ability of the farm's system to detect it) and conception (including successful maintenance of pregnancy). When overall performance does not meet targets, it is often useful to decide which of these two areas should be the focus of attention by assessing measures specific to each element. It is also important to monitor the level of reproductive disease a herd is experiencing so that specific problems can be identified and addressed.

Clear and transparent terminology should be used when defining reproductive performance; this is often hindered by the myriad of performance measurements available. Each has its own advantages and disadvantages, and it is the responsibility of the veterinarian to identify the best way of reporting reproductive performance for each farm. The measurements chosen should reflect the key issues on the farm and allow comparisons to be made with other similar farms. It is not helped when different names are used for the same measurements (see Boxes 25.1 and 25.2).

• BOX 25.1 Risk vs Rate

A rate is the frequency of an event per unit population per unit time. The numerator is the number of events, and the denominator is the animal-time units at risk. This is most widely understood in the context of disease incidence rate: for example, 4 cases of endometritis per month in a herd of 100 cows would equate to an incidence rate of 48 cases per 100-cow-years.

A risk is the frequency of an event, given a designated number of times when it could happen ('trials'). It has no units, as the numerator is the number of cases, and the denominator is the number of trials; e.g., in the herd described previously, assuming all cows calve once in 12 months the risk of endometritis is 48/100 or 0.48.

Reproductive performance metrics can be broadly categorised into interval- or rate/risk-based measures. Many of the risk/rate-based measures are more accurately described as risks, rather than rates (e.g., conception rate). However, across the industry and worldwide it is far more common to refer to rates rather than risks, so to avoid confusion we have used the term 'rate' in this chapter.

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• BOX 25.2 Pregnancy and Conception Rates

Conception rate has commonly been used to describe the risk of a cow becoming pregnant after a given insemination. This is technically inaccurate, as the proportion of cows that conceive (i.e., have a fertilised embryo) is much greater than the proportion that are pregnant by the time of pregnancy diagnosis. Pregnancy rate would perhaps be a more biologically accurate term but is commonly used in other measures (such as proportion of eligible cows becoming pregnant per unit time).

For clarity, in this chapter, we have used conception rate to signify the risk of pregnancy after insemination, and pregnancy rate for proportion of eligible cows becoming pregnant over a defined period of time.

Date*	Br Elig	Bred	Pct	Pg Elig	Preg	Pct2	Aborts
09/11/06	75	33	44	74	15	20	1
30/11/06	72	34	47	70	10	14	0
21/12/06	74	35	47	72	13	18	0
11/1/07	76	46	61	76	14	18	0
1/2/07	76	32	42	76	7	9	1
22/2/07	80	32	40	80	7	9	0
15/3/07	86	23	27	86	9	10	0
5/4/07	80	48	60	78	12	15	1
26/4/07	73	47	64	73	15	21	0
5/17/07	61	36	59	61	16	26	1
7/6/07	45	22	49	44	8	18	1
28/0/07	35	0	0	35	0	0	0
19/7/07	37	2	5	37	1	3	0
9/8/07	64	1	2	64	1	2	0
30/8/07	116	10	9	116	5	4	0
20/9/07	118	90	76	115	33	29	1
11/10/07	94	58	62	0	0	0	0†
1/11/07	72	56	78	0	0	0	0†
Total‡	1168	491	42	1157	166	14	6

• **Fig. 25.1** Example 21-day pregnancy rate report generated using the DairyComp 305 software package (using a 50 day VWP). Br Elig: number of cows eligible to be inseminated in this time window; Bred: number of cows inseminated; Pct: percentage of cows eligible to be served that are inseminated (submission rate); PgElig: number of cows eligible to become pregnant (differs from Br Elig as cows die, leave the farm or are declared barren); Preg: number of cows that actually become pregnant; Pct2: number of successful outcomes achieved expressed as a percentage of the maximum possible at the start of the window (21-day PR); Aborts:number of abortion cases subsequently reported. *Start of the 21-day period †Unknown pregnancy status, awaiting pregnancy diagnosis ‡Note that the totals exclude cows from the last two 3-week periods as the outcomes of these events remain unknown until pregnancy is diagnosed.

Pregnancies per Unit Population Time

'Herd pregnancy risk' is an estimate of the instantaneous probability that an open, eligible cow will become pregnant per unit time in the future. The magnitude of the hazard or risk of pregnancy occurring is determined by the management and environment to which the cow is exposed. In all-year-round calving herds, it is most usefully monitored using the 21-day pregnancy rate (21-d PR) (Ferguson & Galligan 1999, Fetrow et al. 2007). An example of a 21-d PR report generated from the DairyComp 305 software package is shown in Fig. 25.1. For a specific 21-day period, the 21-day PR is calculated as follows:

$$21d\ PR = \frac{Number\ of\ cows\ becoming\ pregnant}{Number\ of\ cows\ eligible\ to\ be\ become\ pregnant} * 100$$

It is important to clearly identify which cows are eligible to be included in the denominator. First, cows are included as eligible if, at the start of the 21-day period for which the PR is being calculated, they had passed their VWP and were not pregnant. Eligible cows also have to have been present in the herd throughout the entire 21-day period (i.e., they must not have been sold or died or otherwise exited the herd), and they must not have been identified as 'not to be inseminated' (and therefore not to be retained in the herd). Finally, ideally all eligible cows should have a pregnancy diagnosis or subsequent insemination that clearly identifies their pregnancy status (yes or no) at the end of the 21-day period. There is some variability between software programmes in how cows are handled which are eligible for only part of a 21-day period; this is important to understand in order to interpret results, especially when rates calculated in different packages are compared. Discrepancies between software packages are commonly accounted for by differences in the way programmes handle cows as they pass their VWP, exit the herd during a cycle, or are inseminated after pregnancy loss.

The related measurement, 21-day submission rate (or 21-day insemination rate), uses the same definition of an eligible cycle, but 'has been inseminated at least once during the cycle' as the outcome; a cow is either categorised as being inseminated (yes) or not (no) during an eligible cycle.

The use of 21-d PR has several advantages over other methods of monitoring reproduction, as the effects of lag, bias, momentum, and variation are minimised (Fetrow et al. 2007). A time lag occurs in all measurements of reproductive performance, as there is an inevitable delay between when a cow is inseminated and when she can be examined for pregnancy. However, by using 21-day PR, this is limited to as short a period as possible so that changes in management and environment that affect reproductive performance are reflected quickly. This means that 21-day PR can be easily used to evaluate and compare different groups and interventions. In reproductive calculations, bias occurs when selection of cows for inclusion or exclusion occurs. For some measurements, only cows currently present in the herd or cows diagnosed as pregnant are included, with cows failing to be inseminated or pregnant being ignored. In contrast, the calculation of 21-d PR includes all eligible cows, whether they were inseminated or not, and includes cows that have left the herd (provided it was after the 21-day period being measured).

A parameter shows momentum when historic data significantly contribute to its calculation. This means that the effect of recent changes (either positive or negative) can be hidden by historic events. As 21-day PR is based on a specific period of short duration, historical data (such as conception rates 60 days ago) have no influence. This does mean that 21-d PR can vary significantly over a short period of time, and that sampling variation can become a problem in smaller herds (see section later in this chapter on Key Epidemiological Concepts). In such circumstances, other measurements of reproductive performance or averaging 21-d PR over a longer time period may be appropriate, even if this increases lag and momentum. Importantly, the short focus of 21-day PR means

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that it is possible for two herds to have similar 21-day PR yet still have different proportions of pregnant cows with a differing pattern of when they became pregnant.

Efficiency of reproduction is influenced by four factors: submission rate (the proportion of eligible cows served per unit time, commonly 21 days); conception rate (the proportion of inseminations leading to a pregnancy); the VWP is and the length of time after calving when it is deemed acceptable to continue inseminating a cow. The latter two factors are both under the direct control of the herd manager. The aim of the VWP, is to provide sufficient time that the chance of pregnancy in an inseminated cow is not compromised by a genital tract that has not fully recovered from the previous calving or a cow in excessive negative energy balance. Typically, on non-seasonal calving farms, cows will not be inseminated (even if they are seen in oestrus) for 35 to 60 days after calving; the exact value depending on the farm situation. On seasonally calving farms the interval between calving and a cow becoming eligible for AI or natural mating is not usually a set period of time, as all non-pregnant cows become eligible for mating when the breeding season starts (usually 85 days after the start of calving).

The acceptable time postcalving when a decision is made to stop inseminating a non-pregnant cow (which will then be culled at the end of lactation) is often established for individual cows, with higher producing individuals**,** those of higher genetic merit, and those with high milk yield persistency being allowed a higher maximum time interval. However, in seasonal or block calving herds, the choice of a maximum interval between calving and insemination is determined at the herd, not the individual, level. In such herd**s**, it is the length of the breeding season that is the key influence on reproductive efficiency.

Once these two parameters are set by management, reproductive efficiency is determined by the proportion of eligible cows that are inseminated per unit time (submission rate) and the proportion of inseminated cows that become pregnant (conception rate). If these two figures are calculated over a 21-day period, the 21-d PR can be estimated by multiplying insemination rate by conception rate.

When calculating average 21-day PR over a longer time period, it is important that it is calculated as a weighted mean of the sum of the pregnancies divided by the sum of eligible cycles (rather than a simple mean of the 21-d PR in each 21-day period). This is particularly important if timed AI programmes are used, as an unweighted mean will tend to underestimate the value of such programmes as it ignores the relative numbers of cows eligible in each 21-day period.

Although the 21-day PR can be calculated from the insemination and conception rates, it can be calculated without any knowledge of either as the only data required are the number of cows which became pregnant during the 21-day period and the number of cows that were eligible to become pregnant during that period. The key advantage of this method of calculation is that it eliminates the need to ensure identical time frames to calculate insemination and conception rates. This metric can therefore also be used effectively in herds relying entirely on natural service by running a bull or bulls with eligible cows.

The 21-day PR approach also provides flexibility as it can be calculated by calendar date (the most common approach), days in milk, days since an event, or days with bull. It can therefore be applied to a variety of herd situations. Fig. 25.2 shows an example of a 21-day PR report calculated by days in milk, with the same data displayed graphically in Fig. 25.3. Comparisons of performance between all-year-round calving herds that operate different VWPs can be made by applying a standard VWP to the calculations for each herd. For example, a herd that operates a VWP of 68 days, because all first inseminations are carried out to a double 'Ovsynch' protocol, could have a 21-day PR of 30% when calculated using the 68-day VWP. This would be equivalent to a 24% 21-day PR for a herd breeding to observed oestrus from 50 days. This again illustrates the point that 21-day PR does not provide information on the pattern of when cows become pregnant, either within that 21-day period or relative to calving.

DIM	Br Elig	Bred	Pct	Pg Elig	Preg	Pct2	Aborts
50	624	612	98	614	197	32	27
71	405	249	61	403	73	18	6
92	322	248	77	318	65	20	9
113	241	159	66	239	47	20	2
134	205	154	75	199	32	16	2
155	176	120	68	173	28	16	5
176	159	106	67	155	23	15	8
197	144	94	65	140	28	20	5
218	113	74	65	110	21	19	4
239	88	62	70	81	10	12	1
260	78	55	71	72	6	8	1
281	74	55	74	71	9	13	1
302	67	49	73	62	11	18	0
323	48	32	67	44	4	9	0
344	41	32	78	34	6	18	1
365	27	21	78	23	4	17	1
386	21	15	71	15	2	13	0
407	14	11	79	10	1	10	1
428	10	7	70	6	0	0	0
449	6	4	67	4	0	0	0
470	3	2	67	2	0	0	0
491	2	1	50	1	0	0	0
Total	2868	2162	75	2776	567	20	74

• **Fig. 25.2** Example 21-day pregnancy rate report by days in milk (explanations as for Fig. 25.1 generated using DairyComp 305).

When comparisons are made between herds, it is recommended that the 21-day PR should be evaluated in all cases using a 50-day voluntary waiting period and that in herds that choose to operate with a different VWP, herd managers should be constantly aware of the 21-day PR; this should be evaluated for both a 50-day waiting period (i.e., with cows becoming eligible for inclusion in the 21-day PR 50 days after calving) and for the actual VWP used on the farm. The former will allow valid comparisons with other similar herds, whereas the latter provides information on the technical success and performance of the programme.

Reproductive performance of a dairy herd is ultimately determined by how rapidly the herd management 'turns open cows in to pregnant cows', a process most accurately measured and monitored using 21-day PR. It is for this reason that 21-day PR has become the standard measure of reproductive performance in many herds.

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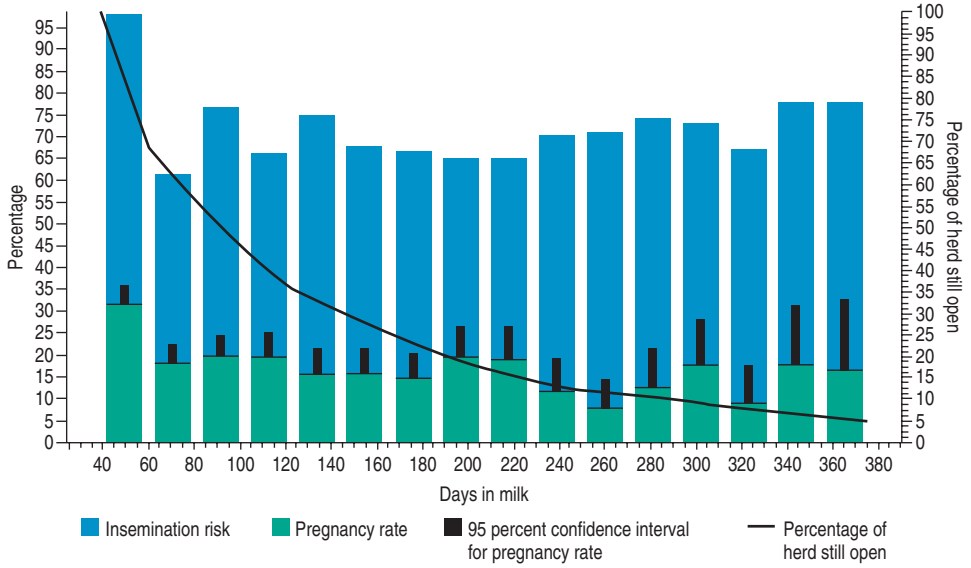

• **Fig. 25.3** Graphical representation of 21-day pregnancy rate by days in milk (generated using DairyComp 305).

Other Fertility Measurements

Measurements of Overall Performance

Traditionally, the focus of fertility has been ensuring the production of one calf per cow per year. This led to the adoption of the calving interval (the interval, usually in days, between successive calvings for an individual cow) as a standard measure of fertility. At the herd level, the average calving interval across a cohort of lactations (e.g., the most recent completed lactation for each cow in a herd at a specific point in time) is referred to as the calving index. The traditionally accepted optimum for calving interval is 365 days, although there is increasing evidence that extended intervals can be just as economically efficient (e.g., see Lehmann et al. 2016).

Irrespective of the optimum value, the usefulness of calving interval as a fertility measurement is limited by its historic nature, which results from the requirement for two successive calvings. This introduces a significant time lag in assessing fertility as there are typically 270 to 285 days between the successful conception and subsequent calving (and thus the calculation of the calving interval). Calving interval is thus a reflection of what happened over 9 months ago, not what is happening currently. In addition, it has two further deficiencies. First, it ignores heifers, which do not contribute to a calving interval until they reach their second lactation. Second, it ignores cows which fail to become pregnant and are culled for failure to conceive. Thus in a herd with a 25% culling rate and a 25% replacement rate, average calving interval will be based on only 50% of the cows in the milking herd.

The calving interval is the sum of two components: the interval from calving to conception and the interval from conception to subsequent calving. The latter component is not really a measure of reproductive efficiency as it is simply the length of gestation and therefore not directly under the control of the herd manager (although this may change with increased availability of quality bulls selected for short gestation length; Jenkins et al. 2016). In contrast, the interval from calving to conception is a reflection of reproductive efficiency as it is a measure of how quickly cattle become pregnant after calving.

The calving–conception interval (CCI) is calculated by counting the number of days from calving to the service that resulted in pregnancy (usually the last recorded service date). So, to calculate the CCI requires three data points per cow: calving date, date of the successful insemination, and a positive diagnosis of pregnancy. To achieve a calving interval of 365 days, the target CCI needs to be 85 days (assuming a gestation length of 280 days). In effect, CCI is simply a way to measure CI with a shorter time lag as the outcome is determined by pregnancy diagnosis rather than by waiting for the subsequent calving, although pregnancy loss can introduce variation between one and the other.

A cow's CCI is composed of two components: the length of the VWP and the number of days between a cow becoming eligible for insemination and becoming pregnant. The first factor is a management decision; the second reflects the reproductive performance given that management decision. In this, it is similar to the 21-day PR. However, a key disadvantage is that, in contrast to 21-day PR, it is an interval-based measurement that does not include data from cows which failed to become pregnant.

The failure of the CCI to include data from cows that failed to become pregnant led to the development of the related measurement: 'days open'. For cows that conceive, days open is equivalent to the CCI, whereas for cows not conceiving, the days open is defined as the time from calving to culling or death, or the maximum interval between calving and insemination.

This difference between CCI and days open highlights the inherent issue with using interval data to evaluate fertility at the herd level, which is identifying the point at which it is possible to collate data and create a meaningful figure. For days open, the definition requires that the measurement is calculated only when all the cows in the group under evaluation have either reached the time after calving when they will no longer be inseminated or have been culled/sold. Although the addition of data from cows that failed to conceive reduces the issue of bias, it increases lag time. In systems in which cows can be inseminated up to 300 days after calving, this can significantly delay fertility evaluation. In contrast, for CCI the definition is more flexible, so it can be calculated earlier; however, if it is calculated too early (i.e., at a timepoint when only a limited proportion of the cohort being measured have conceived), then it will be biased and of limited value. But defining 'too early' is inherently subjective – clearly if

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only 10% of the selected group are pregnant, then calculating CCI is of limited value, whereas if 90% are pregnant calculating CCI is likely to be useful; thus CCI may be useful depending on the farm situation.

The distribution of CI, CCI, and days open is very commonly right (or positively) skewed, i.e., there is a 'long' and/or 'fat' tail to the right of the peak. This can result in the mean interval being significantly influenced by a small proportion of the animals. Days open is typically even more right skewed because of the inclusion of cows culled for failure to conceive, which will all have high values. When evaluating these figures on a herd basis, the median (i.e., the 'middle' interval where the intervals are arranged in ascending order) is therefore generally a more accurate reflection of the performance of the herd (or a typical cow in the herd). It is useful to examine both the mean and the median value as this can give an impression of the degree of skewness of the distribution. Either figure should be used alongside a measure of dispersion, such as the interquartile range, to show how much variability there is in performance between individual cows. Evaluation of the distribution of individual intervals using graphical representations such as histograms and boxplots can provide additional insight. If this approach is being used as a key metric, it can be useful to evaluate days open and CCI at the same time, as this will provide an assessment of performance with and without the potential distorting effect of high values from cows which failed to conceive.

The difficulties in determining when to calculate intervals has resulted in the development of rate-based measurements designed to reflect overall reproductive performance. The most important of these is the 21-day PR, which was discussed earlier, but this is not the only rate-based measure. Morton (2003) described three ratios which are commonly used in Australia and elsewhere to summarise reproductive performance:

1) 100-day in-calf rate: the proportion of cows eligible for rebreeding that conceive within 100 days of calving;
2) 200-day not-in-calf rate: the proportion of cows eligible for rebreeding which do not conceive within 200 days of calving; and
3) 6-week in-calf rate: the proportion of cows eligible for rebreeding that conceive within 42 days of the start of the breeding season.

The first two are used in all-year-round calving farms, whereas the third is designed for seasonal or block calving. Compared with the 21-day PR, the 100-day in-calf rate and 200-day not-in-calf rate have greater lag but may be simpler to calculate and easier for the farm staff to understand. It is important to realise that both measurements, but particularly 100-day in-calf rate, are significantly affected by the choice of VWP. If the VWP is 60 days, then 100-day in-calf rate reflects the success rate during the first 40 days (around two oestrous cycles) after the end of the VWP. If the VWP is then decreased to 40 days, then each cow will have 60 days (around three oestrous cycles) to become pregnant. This is likely to result in an increased 100-day in-calf rate, even though reproductive efficiency has not changed.

Ideally, whichever measurement is used to define overall reproductive performance, it should be used alongside an assessment of the proportion of the herd that were culled due to failure to conceive. If the causes of culling are recorded correctly, then this figure is valuable for interpreting measurements (such as CCI) that exclude cattle that did not conceive as well as determining the economic effect of poor fertility. Unfortunately, in many circumstances such records are not available as the reason for culling is not recorded. Furthermore, especially if only one reason for culling is recorded, the interpretation of the reasons can be difficult; for example, was the cow with a high cell count culled because of the high cell count or because she failed to become pregnant? Ensuring that culls are properly recorded, so that both the timing of the cull and the key reasons for culling (which may be multiple) are known, can be useful for fertility investigations.

If good culling records are not available, then the proportion of the herd being culled after an insemination can be used to estimate the number of cows that have been culled for failing to conceive on the principle that most 'non-fertility' culls will not be inseminated during the lactation as they are already identified for culling. However, there are clearly other reasons why a cow might leave the herd after being inseminated, and the relative prevalence of this will vary from herd to herd and over time, making such proxy measurements difficult to interpret consistently.

Specific Measurements Underlying Overall Performance

For all of the measurements of overall performance, there are two key factors that underlie those figures: i) the interval between cows becoming eligible to be inseminated (either after the VWP or after an unsuccessful insemination) and being inseminated ('submission'); and ii) the risk of an inseminated cow becoming pregnant ('conception').

The most commonly used measurement interval reflecting the speed of submission for first service is the calving-to-first-service interval. This measurement, like CCI, is dependent on VWP as:

$$\text{Calving-to-first-service interval} = \text{VWP} + \text{interval from VWP end to first service}$$

The interval from VWP to first service is determined by when cows first show oestrous activity after the end of the VWP, and also the proportion of oestruses that are detected. The length of the VWP is crucial as, even if all cows are cycling normally and all oestruses are detected, then average calving-to-first-service interval will be, at best, 11 days longer than the VWP (based on an average oestrous cycle length of 22 days). So, if the VWP is 60 days, then calving-to-first-service interval will be at least 71 days. However, in reality the calving-to-first-service interval will be more than 11 days longer than the VWP, as not all cows will be cycling and not all oestruses will be detected.

As with CCI, the calving-to-first-service interval tends to be right skewed so that the median may better reflect herd performance than the mean. It is also important to calculate a measurement of the spread of the distribution (such as interquartile range), as well as the median. Evaluation of the distribution visually (e.g., using a histogram) is also often useful. As with other interval-based measurements, identifying when it can be calculated for a group of cows is not simple.

Alternative rate-based measurements have thus tended to replace the calving-to-first-service interval. The most important of these is the 21-day submission rate (as described in the previous section):

$$21d\ submission\ rate = \frac{Number\ of\ eligible\ cows\ inseminated\ in\ a\ 21d\ period}{Number\ of\ cows\ eligible\ for\ insemination\ in\ the\ same\ 21d\ period} * 100$$

This measurement has the same advantages as the 21-day PR and can be used in the same way to create an ongoing updated rolling average, particularly in herds in which reproduction data is kept on a computer database. The 21-day (or 24-day) submission

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rates can also be calculated for specific inseminations, particularly the first service. First service submission rate (usually defined as the proportion of cows inseminated within the first 24 days after the end of the cow's VWP), like calving-to-first-service interval, will identify how quickly cows are served once they are eligible for insemination. Unlike calving-to-first-service interval, it lacks bias as it includes all eligible cows not just those that have had a first service. It also has less momentum, so it is better at showing trends over time than the calving-to-first-service interval. However, all cows that fail to be served within 21 or 24 days of the end of the VWP are treated the same; in contrast, calculating calving-to-first-service interval and its distribution will provide information on how long these 'failures' take to be inseminated for the first time.

Achieving a high submission rate is dependent on good oestrous detection. Indeed, in some publications, submission rates are referred to as heat or oestrous detection rates. However, especially in the period immediately after the end of the VWP (or start of the breeding season in seasonally calving herds), submission rates are also dependent on the proportion of cows that are cycling normally. If there are high levels of anoestrus and/or endometritis, submission rates can be low even though heat detection is good.

It is therefore important, if submission rates are low to distinguish between poor oestrous detection and acyclicity. Unfortunately, without additional monitoring (such as frequent progesterone testing), establishing the actual heat detection rate is not possible.

From Table 25.3, the true oestrous detection rate equals a/a + b. However, the only data available on most farms are a + c and b + d. Without additional data, it is not possible to know how many of the oestruses detected were false positives (c), or the number of false negatives (b), the cows that have been in oestrus but have not been detected. However, on farms in which veterinary examination of cows not seen in oestrus is routine, the results of such examinations can provide a useful clue as to the role of acyclical cows in depressing submission rates.

This means that apparent oestrous detection rates (and submission rates) can be falsely inflated if farmers or farm staff regularly submit cows for insemination that are not in oestrus. This could easily happen on a farm that heavily focuses on fertility, and as a consequence, the staff are paid bonuses to improve submission rates. One approach to monitoring, which can provide insight into this, is the evaluation of the intervals between successive inseminations.

The commonly accepted range for the normal length of a bovine oestrous cycle is between 18 and 24 days (see Chapter 1), so ideally the majority of interservice intervals will fall within this range. Intervals between 36 to 48 days suggest that one oestrus has been missed, and intervals of 54 to 72 days suggest that two oestruses have been missed.

Evaluation of the distribution of interservice intervals (for a given time period) can provide useful insights into both the rate and accuracy of oestrous detection. Intervals are usually categorised in relation to the accepted normal cycle length (see Table 25.4).

Relatively high proportions of intervals at less than 18 and 25 to 35 days are indicative of inaccurate heat detection (i.e., a problem with cows being served when not in oestrus); more than 10% of intervals in the former category is sometimes used as a threshold for this. A high proportion of intervals in all categories more than 24 days suggest late embryonic or early fetal death; high proportions of intervals more than 35 days tend to suggest a high proportion of missed oestruses. These suggestions should be supported by evaluating the distribution of interservice intervals, alongside other fertility parameters.

A measurement of overall quality of oestrous detection (known as oestrous detection efficiency) is calculated from the percentage of all intervals within each category, as follows (using the categories defined in Table 25.4):

$$ODE = \frac{b+d}{a+b+c+2(d+e)} * 100$$

Recent evidence suggests that in at least some dairy cow populations, 18 to 24 days may not represent the most appropriate choice of the normal range for this type of analysis and that a high proportion of intervals are at 25 and 26 days (Blavy et al. 2016, Remnant et al. 2015). This should be recognised when interpreting interservice intervals and rates derived from them (such as the proportion of returns to service occurring at 18–24 days after a previous insemination, sometimes referred to as returns submission rate).

Measuring the risk of an inseminated cow becoming pregnant is much simpler as this can be calculated from the number of inseminations given (note this is not the same as the number of inseminated cattle) and the pregnancy test results for the inseminated cows. Conception rate can be defined as the proportion of inseminations leading to a pregnancy (see Box 25.2).

The inverse of conception rate is the average number of services per pregnant cow. Conception rate is a very flexible measurement; although it is commonly calculated and presented on a monthly basis, it can be used for any defined group of inseminations. However, similar to 21-day PR, care must be taken when comparing values between groups if the number of cows per group is small. Examples of groups for which conception rate can be measured include: those based on days in milk (which will identify if specific lactational stages are affecting underlying cow fertility); parity (particularly useful for identifying issues with heifers in their first lactation); fixed-time inseminations (FTAI) after synchronisation programmes (in which conception rate is the best measure of the success of such programmes); and number of inseminations a cow has received.

TABLE 25.3 Test characteristics of a farm's oestrous detection

		TRUE STATUS	
		In Oestrus	Not in Oestrus
Farmer record	Seen in heat	a	c
	Not seen in heat	b	d

TABLE 25.4 Commonly used categories of interservice interval

a)	2–17 days	1-day intervals excluded
b)	18–24 days	Normal interval length
c)	25–35 days	
d)	36–48 days	Double normal interval length
e)	> 48 days	

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When accurate pregnancy test data are not available, non-return to service at a specified timepoint (e.g., 24 days or 56 days post-insemination) may be used as a proxy outcome. Although better than nothing, the non-return rate is not a good measurement of the success of insemination because it is dependent on both the conception rate and the submission rate of non-pregnant, but inseminated, cows. Thus a low non-return rate can indicate either good reproductive performance (high conception rate), or poor reproductive performance (low return to service submission rate). The influence of submission on this measurement is dependent on the timepoint chosen – rates of non-return at later timepoints (e.g., 100-day non-return rate) are likely to be more reflective of true conception rates but introduce an increased lag into the metric.

Of the two factors influencing overall reproductive performance, oestrous detection and submission is generally more susceptible to human control than conception rate. On most farms, improving the submission rate so that more cows are inseminated (and thus have the chance to become pregnant) will be more effective than focusing on improving the chance of individual cows becoming pregnant to a single service. Improving the submission rate can result in improved reproductive performance, even if the conception rate is reduced, provided the reduction in conception rate is less than the increase in submission rate. This principle is the basis by which oestrous synchronisation works: more cows are inseminated (often 100% of a group), so more cows become pregnant, even though at the individual cow level the risk of conception may be lower than if the cow was inseminated to an observed normal oestrus.

In addition, it is important to recognise that poor understanding of oestrous detection is an important cause of low conception rates. This occurs when an inappropriately high proportion of cows are inseminated when not in oestrus. This problem commonly goes hand in hand with poor submission rates (i.e., herds that are incorrectly identified in oestrus are also more likely to miss true oestrus), although this is not always the case.

Monitoring Reproductive Disease

Minimising reproductive disease is essential for optimising fertility. In order to do this, it is essential that reproductive disease is clearly monitored and recorded. The reproductive diseases can be divided into those diagnosed by the farmer and those in which veterinary intervention is required for diagnosis.

The key diseases that need to be accurately identified by farm staff are dystocia and retained fetal membranes (RFM) because in most cases veterinary involvement is not necessary. In both cases a clear definition is needed so that, as much as possible, records are consistent within farms (and, if feasible, between them). Dystocia (see Chapters 11–18) can be recorded simply as an assisted calving but ideally should be categorised based on difficulty: (1) no assistance; (2) slight assistance (one person without calving aids); (3) considerable assistance (one person with aids or more than one person); (4) veterinary assistance, including Caesarean operation (Mee et al. 2011). Categorisation will limit the effect of different farmer approaches to dystocia (e.g., interventionist or not); the dystocia risk should be reported as incidence per calving. This is because using time-based measurements, such as cases per cow per year, means that long calving intervals can reduce the apparent rate of dystocia, as fewer cows will calve each year. Comparison between groups can be useful, particularly comparing heifers calving for the first time with older cows, and between animals bred to different bulls, especially when beef and dairy bulls are used on the same farm.

Even though, in the vast majority of cases, treatment of RFM is unnecessary; retention of the membranes is a key risk factor for uterine infection (both metritis and endometritis; see Chapter 25) and thus for future reproductive performance. Recording which cows have had RFM will identify those that need additional observation in the immediate postpartum period, and examination/treatment before being bred, even if there has been no apparent clinical effect. Using consistent definitions is important. The simplest definition of RFM is a cow that retains its membranes for more than 24 hours. This is not necessarily the most accurate definition, but it is easy for farmers to record accurately (Laven & Peters 1996); as with dystocia, RFM should be reported as incidence per calving cow.

Metritis and abortion (see Chapters 23 and 24) need to be identified by farm staff, but recording will be more valuable if there is veterinary involvement. For metritis, veterinary involvement in developing the strategies to identify sick cows in the early postpartum period is likely to make them significantly more effective. This is because there is no simple single clinical sign that will identify metritis, so skilled staff are required to identify sick cows in the immediate postpartum period (Burfeind et al. 2014). Again, metritis should be reported on the basis of incidence per calving cow.

Loss of the fetus after the diagnosis of pregnancy can be a significant problem. Abortion (loss of one or more calves between 152 and 270 days of gestation) can be diagnosed by the farmer if the aborted fetus is identified**.** However, abortion incidents appear to be underrecorded on many farms (Lemire et al. 1993). Fetal loss, from the time of pregnancy diagnosis to 152 days, is commonly not recorded as a discrete event by farmers, although measuring the proportion of cows being re-inseminated after a positive pregnancy diagnosis can be a useful guide (Forar et al. 1995). Thus the true level of fetal loss (and to a lesser extent abortion) can be challenging to estimate in the absence of diagnosis of the pregnancy status.

The simplest measure of abortion frequency is the incidence risk of abortion, expressed as a percentage of pregnancies. This is useful for quick calculations such as identifying abortion outbreaks. However, it is better to assess abortion risk, particularly over longer periods of time, using a days-at-risk model. This is because abortion data is both right and left censored. The data are right censored principally because, once cows have aborted, they stop contributing days-at-risk. Estimating a simple proportion ignores the effect of early abortions; in addition, pregnant cows can be sold, culled, or die. These animals need to be included in days-at-risk and, ideally, pregnancy tested before removal. The data are left censored as the cow is not 'at risk' of detectable abortion until after pregnancy diagnosis has occurred. Early pregnancy diagnosis increases the apparent rate of fetal loss – not only because it increases days at risk but also because most fetal loss occurs early in gestation, and there is a significant negative association between the risk of fetal loss and days pregnant (Forar et al. 1995). Abortion rate per 100 days-at-risk accounts for many of these issues but is more complex to calculate and interpret. Survival analysis, which plots abortion risk against gestational age, is the most useful method as this can take into account differences in the timing of pregnancy diagnosis. The key issue with days-at-risk is identifying when the abortion occurred; to obtain accurate information requires close observation and regular monitoring of pregnancy status. The latter is likely to be feasible only when a problem is suspected as a result of an increased number of apparently pregnant cows failing to calve.

Endometritis (see Chapter 23) is one of the most important reproductive diseases in terms of its effect on reproductive performance

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(Gilbert et al. 2005). Although some cows with endometritis will show a vulvar discharge, the majority will not; thus identifying endometritis needs an active rather than a passive approach (Gilbert et al. 2005). Various methods of diagnosing clinical endometritis have been used, but probably the simplest and quickest to use on a routine basis is the Metricheck device (see Chapters 23 and 24). In addition to being quicker, the Metricheck device causes less discomfort to the cow and has a greater sensitivity compared with vaginoscopy, although specificity is reduced (LeBlanc et al. 2002).

Whatever method is used, routine examination for endometritis should be undertaken before the breeding period, i.e., before the end of the VWP in all-year-round calving herds or the planned start of mating in seasonal ones. Ideally, it should be done at least 3 weeks before intended breeding as, if treatment is successful, the cow should have at least one oestrus before the start of breeding. The timing of the diagnosis relative to calving is important as early diagnosis and treatment improves reproductive outcomes; but cows should be at least 4 weeks postcalving before being treated (LeBlanc et al. 2002). Endometritis frequency is most usefully measured as an incidence risk per cow calved; if a cow has more than one diagnosis of endometritis during a lactation, it should only contribute once to the numerator.

Monitoring Fertility in Nulliparous Heifers

When monitoring reproductive performance, it is important that nulliparous heifers are not excluded. Several studies have demonstrated that replacement heifers calving for the first time at around 22 to 24 months of age are likely to be the most productive and profitable individuals in the herd (Bach 2011, Brickell & Wathes 2011, Sherwin et al. 2016). In order to achieve this, heifers must conceive promptly and maintain pregnancy once they have reached target breeding age or size; breeding from around 13 to 14 months is usually required to achieve the target age at first calving.

Monitoring heifer performance can be done using broadly similar approaches to those described for the milking herd. When record keeping for this group is good and herd policy sufficiently clear and consistent (such that every animal becomes eligible for breeding at a specified age and all insemination and pregnancy diagnosis events are recorded accurately), the 21-day pregnancy rate approach can work well. As with adult cows, the proportion of eligible heifers becoming pregnant per unit time can be evaluated over time or by heifer age. This approach can also be useful when only natural service is used, with service dates unrecorded, but periodic early pregnancy diagnosis performed.

Again, as in adult cows, interval-based measurements can also be useful. In heifers, age at first calving is the most commonly used, but the interval from target breeding age to first insemination can also be useful. In both cases, it is useful to evaluate the distribution of intervals or at least to calculate both the mean and median rather than relying on a single average value. Interservice intervals can also be used, although it is likely that oestrous cycle lengths tend to be shorter in nulliparous animals (Wolfenson et al. 2004). Conception rates can also be analysed in exactly the same way as in cows, although this is usually only practical in herds using AI.

Key Epidemiological Concepts

When analysing any aspect of herd performance data, it is important to keep some basic principles of epidemiology and statistics in mind. Perhaps the most useful is the effect of the size of the denominator population and the potential for sampling variation when the denominator is small. Herd size clearly plays an important role here; in very large herds, it is rare for small denominators to be a practical problem. However, even in herds with hundreds of animals, the denominator for a particular performance metric may still be small. For example, in a herd with good fertility performance, only 12% to 18% of cows are likely to be eligible for breeding at any one time. Thus a herd with 200 cows may only have around 25 eligible for service in a given 21-day period. If around 20% of these are expected to become pregnant, this would represent only five expected pregnancies. Clearly, there is potential for this outcome to be influenced by chance, and one or two more (or less) pregnancies will make a relatively large difference to the outcome being measured. If the analysis is restricted to cows eligible for first insemination or at a specified stage of lactation, then the denominator clearly becomes even smaller.

Some analysis software gives an indication of the uncertainty in an estimate of a particular performance measurement, for example, by providing p-values for contrasts or showing confidence intervals around estimates of proportions. It can also be useful for the size of the denominator to be displayed alongside the result so that the user has some ability to judge empirically the potential for sampling error to be important. There are no 'hard and fast' rules, but the approach outlined in the previous paragraph can be useful (i.e., considering the potential for a small number of fewer or more events to influence the measured outcome to the extent that decision making may be influenced). When this problem is encountered, commonly the most appropriate option is to increase the time frame of the analysis to increase the effective size of the denominator. For example, in the 21-day pregnancy rate example described previously, the 9-week or 12-week rolling average may reflect true performance more accurately than the rate in each individual 3-week period.

In this context, it is also interesting to consider the concepts of type I and type II error. These terms describe the potential errors in making an inference from a given result. A type I error refers to accepting or acting on an apparent trend or association, which is actually due to chance. A type II error is the reverse: failing to accept or act on a trend or association which is real. The probability of making either error is based on the level of evidence the decision maker requires before acting; when this is very strong, type I errors become less likely but type II errors more likely. There is an important contrast here between clinical epidemiology at an individual herd level and epidemiological research. In the latter situation, avoiding a type I error (for example, in promoting a research finding which is illusory) is often much more important, so relatively strong evidence is required in order to take action. When working with a single herd's data to drive herd level decision making, it is usually necessary to act based on weaker evidence, as the cost of failing to act in the face of a genuine trend is larger relative to the cost of acting on false evidence. For example, a p-value less than 0.05 or 0.01 is commonly used to signify statistical significance in a research context – at a herd level, use of this criterion would generally mean that change is rarely advocated.

The Economics of Reproduction

Components of Economic Loss

It is clear that a herd with very poor reproductive performance will suffer economic losses in a variety of areas, and fertility has commonly been cited as a key driver of efficient milk production (Evans et al. 2006, LeBlanc 2007). Decreased milk production is

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the most obvious source of this loss, with longer intervals between calvings leading to cows spending relatively more of their lives in later lactation, when daily production is lower and feed conversion less efficient. However, this needs to be balanced against the fact that cows with shorter calving intervals will spend an increasing proportion of their lives dry and that high culling rates to maintain a short calving interval can also reduce overall efficiency. Reproductive (or 'failure to conceive') culls are the other commonly recognised source of financial loss due to subfertility. Culling cows which fail to become pregnant incurs a cost as the slaughter value of a cull cow is generally substantially lower than the cost of producing or buying a replacement; this is highly variable. In areas where seasonal and year-round calving herds are both common, cows not becoming pregnant during the breeding season are often sold to year-round calving herds, which can decrease the net replacement cost. It is also important to consider the potential increase in herd asset value, when a cull cow is replaced with a heifer. In herds in which replacements are homebred, there is usually a finite pool of animals that can enter the herd per unit time and therefore a fixed maximum number of culls if herd size is to be maintained. In this situation, reproductive culling can utilise replacements that could otherwise be profitably employed replacing culls for poor production or health reasons.

However, there are a number of other mechanisms by which poor reproductive performance can have an economic cost. Although it can be considered that calves are a by-product of dairy production, they are also an income source, whether reared as herd replacements or sold for beef production. Clearly, the more frequently cows calve in a herd, the larger the number of calves produced per year. There are also direct costs associated with reproduction, such as labour, semen, medicines, and veterinary time. However, some costs are much less obvious, and are harder for herd managers to appreciate. For example, a substantial association has been demonstrated between the duration of the calving interval and the risk of a cow being culled from the herd in the subsequent lactation (Pinedo & De Vries 2010).

There are also some less obvious economic disadvantages associated with shorter calving intervals. Decreasing the number of calvings per unit time (either at cow or at herd level) decreases the risk of diseases associated with calving, including mastitis and lameness, as well as reproductive disease. Pregnancy is also associated with a decrease in milk yield, so cows that become pregnant in early lactation would be expected to have lower yields compared with a similar non-pregnant cow. However, this change is relatively small and has only been demonstrated in the later stages of pregnancy (Roche 2003).

Approaches to Estimating Economic Loss

Two main methods have been used to combine the costs described previously and generate estimates of the likely effect of reduced reproductive performance on profitability. The traditional approach has tended to involve estimating the cost per additional day added to a cow's calving interval, based on the principle that each additional day that the calving interval is extended postpones the subsequent lactation by 1 day, thus effectively substituting it with a day at the end of the current lactation (Esslemont & Kossaibati 2002). Cost in milk production is therefore estimated by subtracting the expected daily yield for the additional day at the end of lactation from the average expected daily yield in the subsequent lactation and multiplying this by margin over feed cost. Daily costs can also be allocated to other components of economic loss and added to this to provide an estimate. As with other approaches, estimates derived by this route are sensitive to a variety of inputs that can change markedly over time, perhaps most notably milk price. Therefore especially in areas in which milk price is mostly driven by the global commodity market and is highly volatile, it is of limited value to produce even 'typical' figures that are widely used across the industry. Estimates should be made at herd level and adjusted to reflect changing market situations. An example of such an estimate is given in Table 25.5.

TABLE 25.5 An example of a cost estimation using a 'cost per day' approach

Cost per Day (£)	365–395d	396–425d	> 425d
Lost milk (margin)	1.57	1.79	1.90
Lost calf value	0.25	0.25	0.25
Extra serves and vet treatment	0.70	0.70	0.70
Gain in yield when empty	–0.13	–0.13	–0.13
Reduced disease risk	–0.43	–0.43	–0.43
Total	£1.96	£2.18	£2.29

Estimation of the economic effect of reproductive performance also lends itself well to simulation-based approaches, which have also been used extensively in this field. This approach is much more flexible, can account more appropriately for a wider range of considerations, and is generally considered likely to produce more robust results. However, it is inevitably less transparent, so that the results are more difficult to explain and justify. It is also harder to customise or update estimates derived using this approach to reflect a specific farm situation, although tools (such as that described by Giordano et al. 2012) for this are available. It appears common for both approaches to generate broadly similar estimates under most circumstances, although this may change as simulation models become more complex and realistic. Simulation can also be used to evaluate the economic value of fertility in block-calving systems (Shalloo et al. 2014).

Veterinary Management of Reproduction

Change in the Role of the Clinician

The last 50 years have seen an evolution in the role of the veterinarian in herd reproduction. Initially, the focus tended to be on identification, diagnosis, and management of individual 'problem' cows, such as those observed to have vulvar discharge or abnormal oestrous cycles. Subsequently, the emphasis expanded to cover the examination of clinically normal cows, with a view to implementing management strategies solely to reduce the time to conception. This included routine examination of cows not seen in oestrus by a specific point in lactation or presentation of cows for pregnancy diagnosis to identify and treat non-pregnant animals. Routine fertility visits to carry out these examinations have been a mainstay of farm animal veterinary practice worldwide over the past 20 to 30 years. More recently, the focus has moved on, and it is now widely recognised that the veterinarian should also be able to add significant value to herds in monitoring data and giving appropriate management advice. This is now a core part

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TABLE 25.6 Example of groups of cows selected for examination at routine visits with major differential diagnoses and key features of suggested examination

Reason for Presentation	Example Criteria[a]	Main Focus of Examination	Major Differential Diagnoses
Postnatal examination	• Routine at around 21 DIM • Abnormal vaginal discharge < 21 DIM	• Vaginal examination • +/– uterine ultrasonography	• Normal • Endometritis • Postpartum anoestrus
Oestrus not observed	• No oestrus behaviour observed by 42 DIM • Not inseminated by VWP + 21 days • Not inseminated as expected after previous treatment	• Ovarian palpation/ ultrasonography	• Normal • Cystic ovarian disease • Postpartum anoestrus • Abnormally persistent corpus luteum • Endometritis
Pregnancy diagnosis	• > 30–50 days after last insemination	• Uterine palpation/ ultrasonography • +/- ovarian examination	• Pregnant • Non-pregnant
Repeat breeder	• > 3 unsuccessful inseminations	• Uterine palpation/ ultrasonography • Ovarian examination	• Apparently normal • Identifiable uterine/ ovarian pathology
Unexpected reproductive events	• Abnormal vulvar discharge > 20 DIM • Short or inconsistent cycle lengths • Oestrus behaviour after positive PD	• Determined by reason for presentation	• As appropriate to reason for presentation

[a]Criteria will depend on voluntary wait period, clinician, and farmer preference for pregnancy diagnosis timing and level of veterinary input and should be discussed on commencing visits.

of veterinary involvement in reproduction for many dairy cow clinicians.

Routine Fertility Visits

Routine fertility management visits are used by many dairy herds around the world as an essential element in the process of assisting cows to become pregnant, so that they are retained in the herd for a further lactation. However, in the larger more progressive dairy farms typically found in North America, the routine pregnancy diagnosis examination is not part of the process of optimising pregnancy rates but is seen as a key audit point measuring the outcomes of the processes and systems in place. In these circumstances the regular pregnancy diagnostic examination is used as a means to gather information, validating the current process, and evaluating any changes, either intentional or unintentional, which may have occurred. A regular assessment of the success of the reproductive programme in place is also essential to allow the veterinarian to review compliance with the programme and to prescribe the medicines necessary for the programme to be implemented by farm staff. In this context the regular routine visit is truly part of an integrated herd health system, of which reproduction is only one important aspect. In a successful situation the farmer should gain an improved income that exceeds the cost of implementation; the veterinarian profits from the sale of professional skills. In the North American situation, the veterinarian sits as the judge of the reproductive programme that is in place.

Generally at each visit, cows will have been selected for examination based on an 'action list', often generated by a computer software system using predetermined criteria agreed upon by the herd owner; the categories of animals presented for examination will vary from herd to herd. An example of the groups of cows presented is shown in Table 25.6. For each cow presented, an accurate up-to-date record of all her lactation events should be readily available, and, likewise, all findings and actions taken at the visit should be transferred back to the main records system in a timely manner. For herds using a high degree of planned synchronisation protocols, the only cows presented may be those for pregnancy diagnosis, as preplanned schedules may dictate the enrolment of cows to those protocols; for ease of allocating and planning labour, the work may occur at a different time of the week. In these situations the prescribing veterinarian should have been involved in the planning and setup of the programme and preferably have a clear knowledge of which cows will receive which treatments and on which day.

It is important to understand that a cow appears on an action list because she has fallen outside certain agreed reproduction targets set by the herd management team and therefore may be in a situation in which her profitability is falling to an unacceptably low level. The common practice of a herdsperson editing the 'action list' and removing cows before a visit is therefore to be discouraged as those cows removed from the list are only likely to become even less profitable and ultimately hasten their culling from the herd. A major objective of the reproductive programme should be to ensure that every cow has an equal opportunity to become pregnant and be retained in the herd. Similar thinking should apply to cows presented as 'not seen in oestrus' or that are overdue for insemination; failure to take action will almost always result in a greater delay in the cow becoming pregnant, with the consequent reduction in her profitability. The frequency of herd visits can also have an effect on the speed with which cows become pregnant as more frequent herd checks means that, on average, cows will be examined for pregnancy earlier and action on open cows is taken sooner.

For herds undertaking routine visits, the physical facilities should also allow for calm, quiet, stress-free handling of cows. In situations in which cows must be held in pens away from bedding and feed, it should be for a maximum of only 45 minutes to avoid a significant negative effect on the cow's daily time budget (Grant & Albright 2001). Handling facilities that are appropriate for

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smaller herds will be inappropriate for handling larger numbers of cows; for example, a holding pen and crush may be adequate for a 100-cow herd but not for a 1000-cow dairy. When cow handling operations are located away from cow housing and feeding areas, regular inspection of those areas should still be undertaken to assess any effect they may have on reproductive performance or, indeed, other aspects of herd health.

Cows presented for routine examinations should always be inspected for general attitude and appearance, body condition, cleanliness, and, when possible, locomotion scoring. It is good practice to keep written records of these observations at each visit for future review.

Although it is common practice to examine the reproductive tract and ovaries of cows presented at routine visits in order to formulate a course of treatment based on individual cow findings, there is growing evidence that many problems with oestrous cyclicity that manifest themselves as a number of gross ovarian changes are in fact due to disturbances in follicular growth; these have resulted from metabolic or infectious insults that the cow suffered during the transition phase. Attending veterinarians should also bear in mind that seasonal effects also influence ovarian cyclicity and that, for example, it is normal for the incidence of ovarian cysts to triple during the autumn in the northern hemisphere associated with shortening day length (Nelson et al. 2010).

Monitoring reproductive performance (as described in the previous sections) is also a core element of the routine fertility visit and can be included in a variety of ways. For example, a specific additional meeting or visit could be set up on a regular basis, ensuring that appropriate members of the farm management team are present (including those responsible for high level management decisions, day-to-day management of the herd, as well as any other relevant professionals such as the herd's nutritionist). Alternatively, or additionally, some time could be set aside at the end of routine fertility visits to assess and discuss performance (and, as a result, potential management changes) in a more informal way, with outcomes summarised in a short e-mail or note.

The frequency with which monitoring is carried out depends on herd size and seasonality. Larger herds will accumulate higher numbers for monitoring much more quickly, so they are generally more appropriately monitored on a more frequent basis (for example, fortnightly monitoring may be appropriate for a very large, year-round calving herd). This allows changes to be put in place rapidly in response to a perceived change in performance. In smaller herds, making decisions based on a small denominator population (e.g., measuring conception rate based on a small number of inseminations) risks identifying a false trend or association (see Key Epidemiological Concepts section). This means that monitoring in smaller herds is inevitably more retrospective, with decreased ability to react quickly to changes. In order to minimise the temptation to commit this error, it is often appropriate to evaluate data from smaller herds less frequently. For example, a chi-squared test of a change in conception rate from 40% to 30% (a change of highly clinically important magnitude for most herds) produces a p-value of almost 0.28, when there are 25 inseminations in each group (i.e., 25 at 40% and 25 at 30%). This is roughly the number of inseminations that might be expected in a 100-cow herd in each month; the p-value can loosely be interpreted as suggesting a 28% chance that the difference observed is due to chance rather than to a genuine change in performance. The degree of certainty required in order to justify implementing changes will vary between decision makers and different situations, but including two or three months' worth of inseminations (n = 50, p-value = 0.12; and n = 75, p-value = 0.06) would make this trend much more convincing. Monitoring conception rate should perhaps be justified in a herd of this size on a monthly basis, always using 2- or 3-month rolling averages as the key metric. In contrast, a 400-cow herd serving around 95 cows per month would be able to have a high degree of confidence that a change from 40% in one month to 30% in the next month would reflect a true change in performance; here, monitoring would be justified on a fortnightly or monthly basis.

Even when good quality herd records do not exist, some degree of useful monitoring may be accomplished based on the events that are observed at visits. In this situation the number of positive pregnancy diagnoses should be a key metric. This can be compared with the estimated number required at each visit to represent one pregnancy per cow in the herd per year and gives an impression of the level of overall performance. Again, this should be based on the total number aggregated over an appropriate number of previous visits, based on the size of the herd.

Herd Level Aspects

The first step in improving a herd's reproductive performance should be to employ the monitoring approaches described in previous sections in order to determine whether the focus should be on improving submission rates for insemination (in herds using AI), improving conception rates, or reducing the level of reproductive disease. It is frequently most appropriate to select one of these areas for initial improvement, even when performance is suboptimal in multiple areas; it can be challenging to attempt to improve several aspects at once. Reasons for poor performance in each area and potential management changes that can be implemented to address these are discussed in Chapter 24, but some common problems are highlighted in the following sections.

Improving Submission Rate

Rate of submission for service (in herds using AI) is predominantly determined by two elements: the rate or 'sensitivity' of the farm's oestrous-detection system and the ability of the cows to express oestrus. There are often limited clues from data analysis that can help to distinguish between these as potential reasons for a suboptimal submission rate, but strong seasonal patterns tend to suggest an environmental component. Generally, therefore, the approach to a poor submission rate involves assessing both elements to identify risks, and using judgment to determine which area is most likely to be limiting or whether elements of both should be improved.

Expression of oestrus is dependent on a number of factors. These broadly fall into those relating to the cow's environment and management and those directly relating to the individual cow. A number of environmental factors have been associated with oestrous expression, such as lack of appropriate space (especially lack of loafing space in cubicle housing; Pennington et al. 1985), poor quality or badly repaired floor surface, and high temperature and/or humidity. Management factors such as time budget (which can become limited, for example, when a group is milked three times daily with a long milking time) and group size are also common problems. Of the cow factors, lameness is perhaps the most important and frequently encountered. There is a substantial body of evidence associating lameness with decreased reproductive performance at cow level (Sogstad et al. 2006, Machado et al. 2010), although this effect may be relatively small at herd level (Hudson et al. 2014). High milk yield has also been reported to be associated with decreased intensity and duration of oestrus

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(Lopez et al. 2004), but there is little evidence that this association is mediated by negative energy balance.

The efficiency of the farm's system for detection of oestrus clearly depends on the method or methods of oestrous detection employed. When visual detection is used, frequency and timing of detection alongside training of observer are critical. A number of visual signs have been described, among which observing a cow standing to be mounted by another cow is usually considered the most reliable (sometimes described as the 'primary' sign). It is widely accepted that observation of this sign alone is sufficient to diagnose oestrus. Less reliable (or 'secondary') indicators include mounting other cows (either at head or hindquarters), chin-resting, and vulva sniffing. Van Vliet and Van Eerdenburg (1996) describe a scoring system for visual detection that assigns a number of points to each of the behaviours observed and suggests diagnosis of oestrus when a score threshold is reached. Even when this is not used systematically, this scoring system can be a very useful training aid to help observers rank the relative importance of the various signs. Evidence suggests that modern Holstein cows commonly demonstrate standing oestrus (i.e., standing to be mounted) for an average of around 7 to 8 hours (Sveberg et al. 2011, 2015, Yoshida & Nakao 2005), which means that fewer than three periods of detection spaced evenly through the day may reduce effectiveness substantially. Use of information can also be useful in aiding visual oestrous detection. A number of systems can be used to remind farm staff which cows are likely to be in oestrus on which days (i.e., those 19–26 days postinsemination); recording 'reference' heats (when a cow is observed in oestrus during the VWP, so not inseminated); allows anticipation of an 'expected' oestrus date for the cow's first eligible cycle.

A number of oestrous detection aids are available, and are widely adopted in most major dairying nations. The simplest of these involve simple physical approaches to detecting when a cow has been mounted by another cow (and is therefore assumed to be in standing oestrus). These devices include tail paint or chalk, self-adhesive scratch cards, and capsule-type systems (see Chapter 24). These have various strengths and weaknesses, and there is no compelling evidence to support use of one over the other. In practice, it appears that one or the other commonly works better in a particular farm situation. Reported sensitivities of these systems vary widely (probably because of differences in cow and environment factors between studies, which are very commonly based on a single herd); they range from around 35% (Holman et al. 2011) to over 90% (Xu et al. 1998).

A more sophisticated (and more expensive) approach is the use of sensor technology, either as a standalone system or integrated with milking parlour or voluntary milking systems. Activity monitoring is by far the most common sensing approach used for oestrous detection, and has been relatively widely adopted since the mid-2000s. These systems detect an increase in activity (usually via an accelerometer attached to a leg or neck collar), as cows move about more when demonstrating oestrus. An increase in activity beyond a specified threshold above that expected triggers an alert that the cow may be in oestrus. As with other methods, a range of test characteristics are reported in the literature. Reported sensitivity values are typically in the range 60% to 90%, with positive predictive value generally 70% to 95% (Firk et al. 2003, Holman et al. 2011, Roelofs et al. 2005). Both the algorithms for interpretation of activity data and the sensors themselves are continuously being improved, so it is likely that this method will become increasingly effective in future.

Implementing a combination of more than one method is often associated with the highest levels of submission rate, plausibly because different methods detect different oestrous behaviours. Milk progesterone testing can also be useful (greatly facilitated when tests can be done quickly at cow side). It is especially useful when used in parallel with a measurement that would be expected to increase sensitivity but decrease specificity of oestrous detection; for example, reducing the threshold required to trigger an alert on an activity monitor system. Alerts associated with activity levels above the previous (higher) threshold could still be used as a basis for insemination with no further evidence, and alerts between the new and old thresholds screened using milk progesterone, thus inseminating cows when the progesterone level is low. This often detects a higher proportion of oestruses without the drawback of inseminating more 'false positive' cows. Effective use of veterinary examinations and fixed-time artificial insemination protocols can also be extremely helpful in improving submission rates; these are discussed elsewhere in this chapter.

Improving Conception Rate

As with submission rate, there are a wide variety of possible causes of poor conception rate. Nutrition is one of the most commonly implicated areas; there are a number of possible nutritional factors that may influence conception rate. Perhaps the most common is excessive negative energy balance (NEB) during early lactation (see the review by Roche (2006)). Analysis of body condition score data and estimation of blood ketone and non-esterified fatty acid concentrations can be very helpful in quantifying postcalving energy balance and evaluating the potential importance of this aspect for a particular herd. Energy balance may also play a role in mediating the association between conception rate and temperature and humidity, a key environmental factor influencing conception rates. Micronutrient nutrition can also influence conception rates, although in most systems, this is uncommon compared with the effects of negative energy balance. When NEB is the key factor, data analysis can reveal lower conception rates in early lactation or in higher yielding cows; if micronutrients are involved, conception rates tend to be poor across all inseminations. The role of subacute ruminal acidosis in compromising conception rates remains controversial.

A number of diseases are also associated with reduced conception rates. Probably the most important (in this context) are the postcalving reproductive diseases, including retained fetal membranes, metritis, and endometritis. These are strongly associated with decreased fertility and should always be considered when a high rate of such diseases is observed in conjunction with poor conception rates (especially when conception rate is particularly low in early lactation). Herd level infectious diseases, such as bovine viral diarrhoea, infectious bovine rhinotracheitis, leptospirosis, and Johne's disease, have also been associated with decreased conception rates (see Chapter 24). The evidence for this is especially clear when disease enters a previously naïve herd; the size of association tends to be much smaller and the evidence more equivocal for the effect of such a disease in a herd with a moderate level of endemic infection. Non-infectious endemic diseases, such as mastitis and lameness, have also frequently been associated with reduced conception rates in individual cows but are probably a relatively rare cause of a poor conception rate at herd level (Hudson et al. 2014, 2015). Some venereally transmitted diseases (such as campylobacteriosis) are associated with decreased conception rate in herds using at least some natural service.

Finally, there are a number of factors associated with insemination that can result in disappointing conception rates. For artificial insemination, these include poor semen quality or storage, thawing

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and handling, as well as inappropriate timing of insemination relative to signs of oestrus, and inaccurate heat detection (i.e., insemination of cows outside of true oestrus). The latter problem can sometimes be identified by a high proportion of interservice intervals of 'unexpected' duration (i.e., not multiples of the expected normal oestrous cycle length), especially intervals less than 19 days. Cow-side milk progesterone testing may be a useful tool here; for example, measuring progesterone in the next 50 cows to be inseminated allows calculation of the positive predictive value of the herd's heat detection (i.e., proportion of apparent heats that represent a true oestrous event). When natural service is used, establishing the semen quality of the bull team is important, alongside factors such as the ratio of bulls to eligible cows and general bull health.

In herds using natural service alone (where a bull is running with the eligible cows at all times), it is rational to assume that conception rate is the main limiting factor for fertility in most situations, provided that the bull has been observed to have a normal level of libido and good physical mobility.

Minimising Reproductive Disease

A strong association exists in dairy cows between the incidence of inflammatory and metabolic diseases in the first 2 months postpartum and reduced fertility and pregnancy loss during the subsequent lactation (Santos et al. 2010). Calving-related disorders and those that affect the reproductive tract are major contributors to anoestrus or the occurrence of abnormalities of ovarian cyclicity diagnosed later in lactation such as ovarian cysts and anoestrus. It is estimated that approaching half of all cows experience one or more calving-related health disorders in the first 60 days postpartum. Almost all dairy cows are known to experience bacterial contamination of the uterus in the first 2 to 3 weeks postpartum, which, along with the necessary repair of the endometrium during involution (see Chapter 7), means that some degree of uterine inflammation is necessary and is indeed a normal and necessary component of involution (Sheldon et al. 2009). The innate immune system is the main protection for the uterus**,** which may be overcome by overgrowth of pathogenical bacteria**,** or the severity and duration may become excessive resulting in the impairment of future fertility. The high incidence of disease is attributable in part to the reduced innate immune function and regulation of inflammation that occurs in all cows from approximately 2 weeks prepartum to 3 weeks postpartum (Kehrli et al. 1999). However, the metabolic adaptations of insulin resistance (IR) and NEB also contribute to the reduced immune defence. Homeorrhetic adaptations to lactation require that every cow experiences a degree of NEB and IR to support lactation, but as with uterine inflammation, the concern is for cows that experience a severity of these states that goes beyond the merely adaptive to become a contributor to disease and impaired reproduction. The point at which reproductive tract inflammation and metabolic status shift from the physiologically normal to the undesirable is likely to involve a number of multifactorial interactions; these remain poorly quantified and understood. It is well known that during the transition period the dairy cow is at particular risk of the effects of NEB, and it is intriguing that it seems likely that the metabolic milieu that accompanies transition may be responsible for the initiation of inflammation without the presence of a pathogen.

Although relatively little is known about how reproductive tract disease may be influenced by management, it is known that cows that develop severe metritis will have eaten less than normal cows in the 2 to 3 weeks preceding the onset of the disease; reductions in feed intake are associated with increased circulating concentrations of NEFAs, which may indirectly or directly inhibit neutrophil function (Hammon et al. 2006, Huzzey et al. 2007, Ster et al. 2012). The high degree of oxidative stress also experienced by early lactation dairy cattle also contributes to a proinflammatory state that may not be helpful for immune defence mechanisms (Sordillo & Aitken 2009).

Based on current understanding, there are few management practices that can be recommended with certainty that will prevent inflammatory reproductive tract disease in the postpartum period. Current understanding suggests that the objective should be to support and maintain innate immune function to prevent the risk that the normal postpartum bacterial contamination and inflammation does not progress to infection and disease. Suggested management practices generally recommended for peripartum cows that may reduce the incidence of postpartum reproductive tract disease are shown in in Box 25.3.

The aetiologies of cystic ovarian disease and anovulatory anoestrus and associated risk factors are generally considered to be similar to those associated with uterine disease, although less well understood (see Chapter 22).

The causes of abortion are generally much better understood and can broadly be categorised as infectious (including neosporosis and brucellosis) and non-infectious (including stress and exposure to fetotoxic agents). These are covered in more detail in Chapter 24. From a herd perspective, it is important to remember that a certain level of abortion is expected; levels higher than 3% to 5% are commonly considered to merit investigation.

• BOX 25.3 Suggested Management Practices to Reduce the Risk of Reproductive Tract Disease

- Dry cows off to ensure a target dry period length of 35 to 60 days for at least 85% of the herd.
- Prevent overconsumption of energy above requirement during early part of the dry period.
- Provide unrestricted feed access at all times for all dry cow groups.
- Ensure feed is provided ad lib, and all cows can eat at the same time when fresh feed is delivered.
- Provide a minimum of 75 cm of utilisable trough space per cow.
- Provide at least 10 cm of water trough space per cow, sited at a least 2 points in the pen.
- Provide adequate cow comfort with cubicles or open yards to ensure at least 11 to 12 hours lying time per cow per day – at least 1 cubicle per cow and for open bedded areas at least 10 m^2 per cow plus an additional 1 m^2 per additional 1000 kg of milk production over 10,000 kg.
- Minimise group and social changes. Aim to restrict grouping changes to a weekly event at most; ideally use an 'all-in/all-out' system to maintain the same social group over the dry period.
- Ensure each cow spends a minimum of 10 days exposed to an appropriate precalving ration.
- Dry cow and fresh pens should have sufficient capacity to cope with variations in calving pattern. Facilities should provide 130% of average monthly capacity in year-round calving systems.
- Ensure excellent ventilation of dry and fresh cow buildings. If necessary, provide heat abatement, fans, and sprinklers for when the temperature humidity index (THI) exceeds 68.
- Manage overall nutrition for target body condition (BCS) at calving of 3 to 3.25 (on a 5-point scale) and maintain a minimum of 2.5 with no change from dry off to calving.

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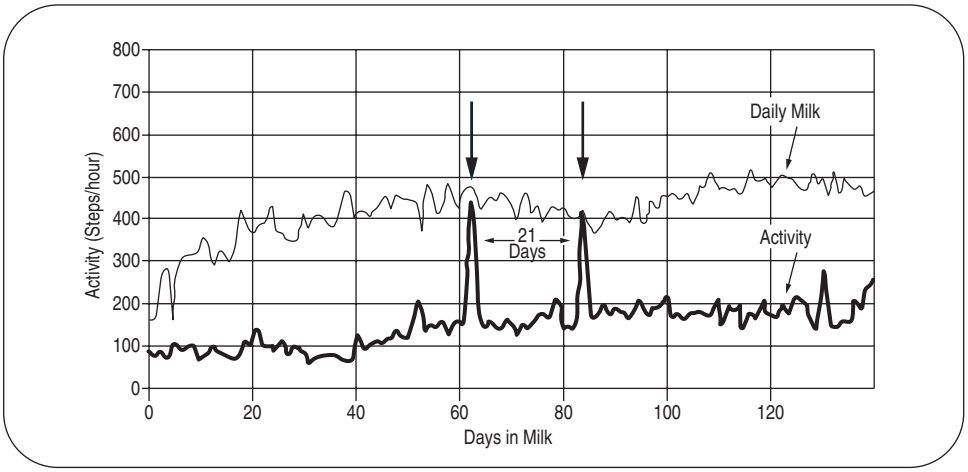
Reproductive Protocols

A number of reproductive management protocols, or controlled insemination programmes, have been developed over the years to meet the need of larger dairy herds. As discussed earlier, on many farms, especially in larger herds, hormone-based programmes such as Ovsynch are being increasingly used so that insemination of eligible cows becomes part of the weekly routine and does not interrupt the normal daily workflow of the dairy unit. However, it is the authors' opinion that an effective oestrous detection system should still be pivotal in an integrated approach to dairy herd reproductive management and should be part of a normal, fixed, daily routine and carried out in a manner specified in written protocols. For example, if a system of tail chalking or tail painting is used, it should be clearly understood by all personnel. Likewise, when telemetric systems of heat detection are in use, data gathered must be checked at least daily so that cows can be inseminated within an appropriate time window (Fig. 25.4).

The protocol chosen depends on the time and skills available on the farm to continue to detect oestrus. Programmes that rely on inducing CL-regression, such as those utilising double or multiple injections of prostaglandin, require an element of oestrous-detection to produce acceptable results and are best described as oestrous synchronisation programmes. Variations on programmes that utilise induced CL regression include systems in which a veterinary examination to identify the presence of a CL, either by manual palpation or ultrasound, is done before injection and that the reproductive tract is healthy; this is a system commonly used in smaller herds. A surprisingly large number of herds still simply administer $PGF_{2\alpha}$ every 2 weeks to non-pregnant cows and simply observe for oestrus. This system can work well if a CL is present and is an easily administered programme requiring minimal record keeping; it can be used for cows and heifers. Good oestrous detection is essential for success. Veterinary examination may be seen as an advantage or disadvantage in these programmes as there are unnecessary costs incurred in examining large numbers of cows that do not require examination. In contrast, protocols that rely on induction of ovulation (such as Ovsynch and related programmes) are much more likely to produce better results to TAI without oestrous detection. Such synchronisation protocols are most often used to ensure timely insemination of cows by a target 'days in milk' to ensure every cow receives a first insemination at a time in lactation that allows her the opportunity to become pregnant and retain her place in the herd. Cows found not pregnant can also be enrolled on to synchronisation protocols to ensure timely reinsemination. In some herds that operate very intensive reproductive programmes, the first GnRH of the Ovsynch protocol can be given to all cows 7 days before pregnancy examination without terminating the pregnancy and so shortening the time to reinsemination by 7 days, a practice commonly termed 'Resynch'. It is worth noting that outcomes from re-synchronisation protocols may be poorer when the interval from the preceding insemination is less than 30 days (Fricke et al. 2003). Perhaps it should also be noted that in herds in which oestrous detection is excellent, aggressive resynchronisation may actually lengthen days open as the initial GnRH treatment may interfere with normal folliculogenesis, thus delaying normal oestrus and ovulation.

The success of these programmes is heavily dependent on compliance with the injection schedule, which requires a high degree of staff skill and training. In a three-injection programme, if compliance is 90% (such that 90% of the cows get the correct treatment on the correct day), only 73% ($0.9 \times 0.9 \times 0.9 = 0.73$) of the cows will complete the programme correctly. Programme compliance can be checked based on audits of the amount of drug used versus cows injected and/or by periodically sampling some cows on either day 7 of a programme or on the day of insemination for milk progesterone measurement.

For herds that use a combination of oestrous detection and synchronisation to ensure that each cow receives a timely first insemination, the question arises as to the 'days in milk' when cows should be enrolled on the synchronisation programme, with most herds opting to ensure that every cow receives a first insemination by 75 to 80 'days in milk'. The rationale for this timing is based on the information that cows that show an early return to cyclicity and an early first ovulation postpartum are likely to be inseminated to a naturally occurring oestrus before this time and conceive earlier than those that show a delayed return to cyclicity and a delay in the first ovulation postpartum (Galvão et al. 2010). The major factors contributing to the absence of cyclicity observed in some cows are the calving-related disorders of the reproductive tract. The number of cows failing to resume cyclicity by 50 to 65 days in milk in many herds is in excess of 20% (Santos et al. 2010). Thus the percentages of cows requiring synchronisation to achieve a first insemination by this time can be used as a crude assessment of health at calving. In herds that combine oestrous detection with synchronisation to achieve their first insemination by 80 days in milk, it is suggested that a target of no more than 25% of first inseminations should be to synchronised inseminations.



• **Fig. 25.4** Example of the output from a simple on-farm pedometer activity monitoring system.

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Most synchronisation protocols used in cows (with the exception of periodic injections of $PGF_{2\alpha}$) are unsuitable for use in heifers due to the difference in follicular dynamics. Addition of an intravaginal, progesterone-releasing device will significantly improve conception rates for fixed-time insemination of heifers (see Chapter 8 for further details).

Routine synchronisation of heifers has the same ethical and welfare issues as routine synchronisation of lactating cattle, although the reasons for its use are generally different – namely difficulty in organising oestrous detection in the heifer group versus underlying poor oestrous behaviour in lactating cows. However, in contrast to lactating cows, synchronisation of heifers can have welfare benefits for the treated animals as the compact calving pattern produced allows the creation of heifer groups that, on larger farms, can be kept together after lactation or, even on smaller farms, carefully managed to minimise the effect of the transition from pregnancy to lactation (Boyle et al. 2013, Neave et al. 2017).

It should always be borne in mind that, despite the use and development of sophisticated controlled breeding programs, ultimately herd reproductive performance will remain dependent on and restricted by the overall standard of the management of health and nutrition of the herd. Pregnancies resulting from controlled breeding programmes will still be subject to the same level of embryo loss and abortion as those resulting from insemination to spontaneously occurring oestrus. Ultimately, success will depend on an integrated approach to fertility management, with the use of synchronisation protocols being just one aspect of a sound reproductive management programme.

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26

Veterinary Control of Herd Fertility in Pastoral Dairy Herds

RICHARD LAVEN

The main features of the annual reproductive calendar are illustrated in Table 26.1 (Holmes et al. 2002).

For a spring calving herd the aim is to have cows calving over a relatively short period during late winter/early spring. They will then be rebred approximately 3 months after the start of calving. The planned start of mating is a set calendar date, designed to result in cows starting to calve at the correct time in the next year. So, in a Northern hemisphere system, where the aim is to have cows starting to calve in the beginning of February, the planned start of mating (PSM) needs to be set 282 days before then (i.e., a PSM date of 27 April for a calving period beginning 3 February).

As mating starts on a specific date, cows become eligible for breeding at a range of postpartum intervals; there is no set voluntary waiting period (VWP). The length of the mating period determines the length of the calving period, so for a compact calving the recommendation is to have a breeding season of 12 weeks or less. However, on farms with moderate to poor fertility, a 12-week breeding season would result in too high a proportion of cows that have failed to conceive ('empty' cows) and that will need to be culled at the end of the season. The length of the breeding season is thus a compromise between the duration of the calving period next spring and the 'empty rate' at the end of this year. This can have positive consequences – with good fertility, the breeding season can be reduced to less than 12 weeks as the target 'empty rate' can be obtained before the 12-week target. The tighter calving pattern that this facilitates brings benefits both in staffing and activity management and in production efficiency (by matching stage of lactation more closely to grass growth and quality).

For the standard 12-week breeding season, the most common practice is to have 6 weeks of AI, usually to bulls of dairy breeds, so that all cyclical cattle can have at least two opportunities to become pregnant. This is usually followed by 6 weeks of breeding with sweeper bulls, which can be extended if the proportion of cows that show heat when the bulls are introduced is higher than expected. On Australasian farms, pregnancy testing has traditionally been undertaken approximately 6 weeks after the bulls have been removed. However, this is now changing, with more herd pregnancy testing at approximately 6 weeks after the end of AI, followed by retesting of cattle not diagnosed as pregnant 6 weeks after the end of the breeding season. The additional early pregnancy diagnosis allows fetuses resulting from AI to be accurately aged, so that the success of the AI breeding period can be properly monitored. In contrast to all-year-round calving herds, cows that are identified at any stage as being non-pregnant are not treated or reinseminated. Once confirmed as non-pregnant, cows remain in the milking herd until they are culled, which, provided grass is available, is usually at the same time as the pregnant cows are dried off.

In New Zealand, drying off time is dictated by the availability of pasture (which is weather dependent) or cost effective supplementary feeds (such as forage crops and palm kernel extract). Most farms in New Zealand aim to dry off cattle as late in the autumn as possible, but this still results in an average lactation of 275 days with a dry period length of approximately 90 days (McDougall 2010). This is because cows tend to be dried off as a group or herd rather than individually, based on expected calving date. In addition, early dry off before the main date is often used as a management tool to preserve grass, reduce bulk somatic cell count, and aid the management of body condition, further reducing average lactation length.

In Australia, where the cost of supplementary feeding with grain is much less than in New Zealand, dry period lengths are shorter and tend to be based on calving date; even in Tasmania, which has the most New Zealand-like system, average lactation length is approximately 300 days (Anon 2015). In Ireland, where dry cows are commonly housed and fed grass silage, dry period length is shorter than in New Zealand but still not as short as would be recommended based on calving date; Cummins and associates (2016) reported that 79% of spring calving herds had an average dry period length between 8 to 12 weeks.

In Australia and New Zealand calving takes place at pasture, with the aim of calving approximately 90% of the herd within 6 weeks of the planned start of calving. In Irish-style systems, in which cows are housed to prevent the effect of winter grazing on spring grass growth, cows should calve before turnout, with a lower target of having 75% of cows calving within a period of 6 weeks from the commencement of calving.

Key Issues Affecting Reproductive Performance

Body Condition Score

In any dairy system, the balance between energy intake/output and fertility are closely linked. In a pasture-based system, there is not the option to alter the diet to increase energy intake. Energy

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TABLE 26.1 Annual calendar of suggested main managemental and reproductive events in a spring calving, pastoral dairy herd

Late Winter/Early Spring

- Cows calving
- Pregnant cows on restricted grazing; may be supplemented with forage crops (such as fodder beet) or grass/maize silage
- Calved cows on unrestricted grazing
- Yearling heifers starting to show significant oestrous activity

Late Spring

- All cows should have calved within 8–10 weeks.
- Oestrus observation starts at least 31 days before planned start of mating. All calved cows tail painted at this time.
- Normal cows should show oestrus within 40–50 days of calving. Anoestrous cows identified and treated before the start of the mating period.
- All cows examined and treated for endometritis before start of premating oestrus observation period.
- Planned start of mating is 83 days after planned start of calving. Aim to breed all cows to AI at least once in the first 6 weeks of the mating period.

Summer

- All cows and 15-month-old heifers should be mated by midsummer.
- Pregnancy test cows and 18-month-old heifers.

Autumn

- Dry off once pasture becomes scarce and/or milk production declines to uneconomic levels.
- Adjust feeding of dry cows to ensure cows calve at correct condition score. Separate thin animals and feed more generously.

Derived from Holmes et al., 2002.

intake is dependent on pasture availability and pasture quality, which are both dependent on pasture management. Furthermore, it is not just current pasture management (e.g., current rotation length, grazing heights, and residual dry matter) that influences pasture quality and availability; it is also past management, especially during the spring season. Poor pasture management during the dry period (i.e., winter) will significantly reduce pasture growth in the spring. The difficulty of feeding the dry cow in winter from grazed grass and maintaining optimal grass growth in the spring is the key driver for housing of the dry cow on Irish pasture-based farms and also the main reason why many dairy farms in New Zealand winter their cows away from the main farm.

Body condition score (BCS) is the best method of monitoring energy balance in adult dairy cattle. In grazing cattle, as in cows fed a total mixed ration, the expectation is that in early lactation there will be a dip after calving as the cow mobilises body tissue to produce milk (Fig. 26.1), with BCS reaching a nadir before the planned start of mating. If BCS is not managed effectively, then fertility will suffer. The key targets are BCS at calving and the loss of BCS between calving and mating. In order to meet the BCS targets for calving, BCS needs to be closely monitored in late lactation so cows can be fed during the early dry period to meet the BCS target.

There are strong links between BCS and reproductive performance, with BCS at calving and/or mating tending to be positively associated with performance and increased loss of BCS being negatively associated (Roche et al. 2007). In seasonally calving herds, the most important effect of low BCS and of excess BCS loss is probably their effect on the postpartum anoestrous interval. This is because the short breeding season means that any delay in returning to oestrus, particularly in late calving cows, reduces the chance of a cow becoming pregnant to AI and increases the chance of a cow failing to get pregnant at all. Interestingly, for BCS at mating, though not at calving or nadir when the relationship was a simple linear one, Roche et al. (2007) found a curvilinear relationship with the odds of oestrus being observed before the planned start of mating being greatest with a BCS of 5.5 (on a 1–9 scale).

Calving Pattern

Attaining a compact calving pattern by inseminating and getting cows pregnant early in a short breeding season is essential for optimising profitability in a seasonally calving pasture-based system. However, the current year's calving pattern has a major effect on the likelihood of getting a compact calving next season. If the current year's pattern is compact (60% calving within the first 3 weeks, and 90% in the first 6 weeks), then it is relatively easy to maintain a compact calving pattern. In contrast, if calving is extended over a long period or large numbers of cows calve after the first 6 weeks, then it can be difficult to achieve a compact calving period in the next year without a high replacement rate.

The effect, like BCS, is principally mediated through return to cyclicity. Cows that calve later in the season will have shorter intervals from calving to planned start of mating than cows which calved earlier. This has two effects: first, there will have been less time to complete involution and restore normal uterine and ovarian function; second, they are more likely to be in negative energy balance. Both of these effects combine to result in later calving cows being more likely to show no heat before the planned start of mating; consequently, later calving cows are less likely to conceive to AI and less likely to become pregnant at all.

The calving pattern also affects the age at which nulliparous heifers are first bred. As with the milking herd, heifers become eligible for mating on a calendar date, not on an age or weight basis. This ensures that heifers calve at (or, more commonly, somewhat before) the same time as the main herd. If the herd has a compact calving pattern, then heifer replacements will be born over a short period of time (often not much more than a month); in contrast, if the calving pattern is extended, then the variability in age between heifers will be greater. In the latter case, if the heifers are all bred at the same time, some heifers will calve at 21 to 22 months of age and will be smaller and lighter and therefore more likely to have delayed return to cyclicity and an increased risk of failing to become pregnant during the breeding season. Delaying insemination until a heifer is 14 months of age will reduce the interval between her first parturition and the start of the subsequent breeding season, again increasing the risk of failing to become pregnant during the breeding season. In New Zealand, most heifers are bred to a small stature bull, usually a 2- to 3-year-old Jersey bull, reducing the need for management around calving. This reduces the rate of genetic gain as replacements are not produced from the animals with the best genetic merit. Furthermore, as well-grown heifers have high fertility and will get pregnant more quickly than well-managed lactating cows, producing at least some replacements from heifers will produce a better-matched age group than producing them from mature cows alone.

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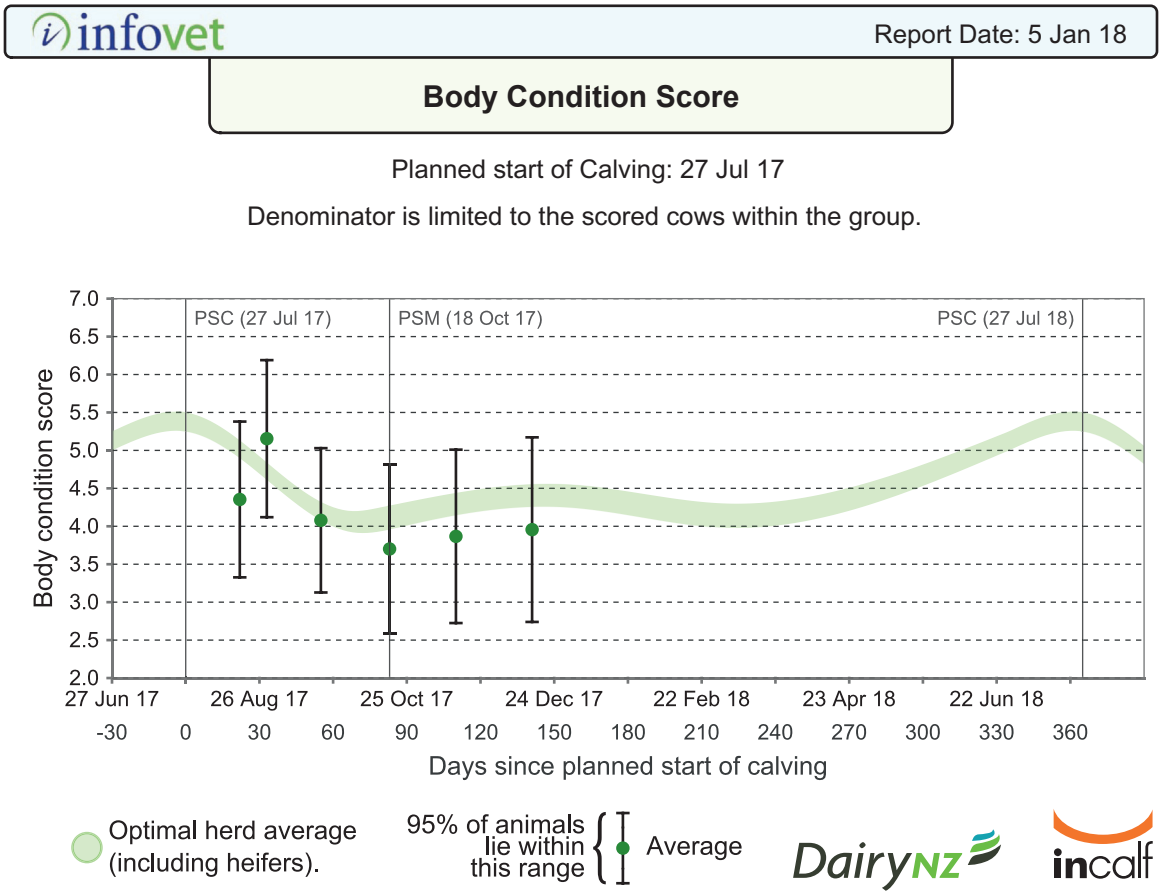

• **Fig. 26.1** Recommended body condition score targets relative to planned start of calving (PSC) and planned start of mating (PSM) for pasture-based cattle in New Zealand. BCS scale is 1 to 9 scale; to convert to Irish 1 to 5 scale: IRE = 0.81 + 0.4 NZ.

As stated earlier, a poor calving pattern is the biggest barrier to improvement in reproductive performance on seasonally calving farms. Culling late calving cows is an essential part of improving calving pattern, but is not sufficient on its own; the culled cows need to be replaced by cows that calve early in the calving season. The best way of doing this is by increasing the number of heifers kept as replacements. In a herd in which the replacement rate is normally 25%, improving the calving pattern will need at least 30% of the herd needs to be replaced annually by early calving heifers.

Heifer Management

Rearing heifer replacements is one of the biggest costs on a dairy farm, so it is essential to produce quality heifers that will have long and productive lives, which necessitates good reproductive performance in their first and subsequent breeding seasons. Monitoring and meeting bodyweight targets is crucial if this goal is to be met. On many farms the targets (Table 26.2) are set based on the average mature live weight in the herd, but with good quality genetic data and computerised systems, the targets can be set at the individual cow level (Roche et al. 2015).

Regular monitoring, ideally monthly, is essential. First, simply meeting the final 22-month target does not prevent the effect of earlier poor growth. Heifers that fail to meet the breeding target but that are closer to achieving the precalving bodyweight target are often small and fat, increasing the risk of dystocia and of future reproductive problems. This can be identified by measuring stature and BCS alongside live weight. Second, regular monitoring allows early intervention either at the group or individual level. Consecutive weight measurements can be used to calculate the rate of weight gain, and plotting this against current weight will allow heifers to be categorised into (Fig. 26.2): i) On track: weight gain and current weight better than target; ii) Recovering: current weight less than target but weight gain better than target; iii) Monitor: current weight more than target but weight gain below target; and iv) Action: current weight and weight gain less than target.

TABLE 26.2 Recommended targets for live weight for pasture-fed heifers

Age	Target
3 months (weaning)	20% of mature live weight
11–12 months (puberty)	50% of mature live weight
14–15 months (breeding)	60% of mature live weight
22 months (precalving)	90% of mature live weight

Anovulatory Anoestrus

In seasonal systems all non-pregnant cows become eligible for mating once the breeding season has started. This means that cows that

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Animal performance

All 88 animals in this weighing are displayed

Take action with these animals

Official Id	AE Breed	Current Weight (Kg)	Weight Gain (Kg/day)	Gain Required by PSM (Kg/day)	Variation from Ideal (%)	Previous Category
330	H/Friesian	240	0.80	1.20	-16.22	Recovering
341	H/Friesian	196	0.80	1.64	-29.27	Action
CCVK--18	H/Friesian	233	0.75	1.05	-13.84	Action
CCVK-43	H/Friesian	172	0.71	1.95	-37.84	Action
CCVK-11	H/Friesian	230	0.68	1.25	-18.10	Action
CCVK-1-74	H/Friesian	237	0.70	1.19	-16.23	Action
CCVK-1-106	H/Friesian	211	0.75	1.39	-22.86	Recovering
CCVK-1307	H/Friesian	191	0.70	1.60	-29.32	Action

Showing 1 to 8 of 8 entries

• **Fig. 26.2** Example graph showing whether current weight gain and current live weight is meeting target (based on © MINDA, LIC, www.lic.co.nz.).

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calve more than 6 weeks after the start of calving, have less than 40 days to return to normal cyclicity. Furthermore, cows with any reproductive abnormality, such as dystocia or endometritis, may have only a short time to restore normal function before the start of the breeding season even if they calve in the first 6 weeks. It is thus unsurprising that a high proportion of cattle in such systems (around 20%; Rhodes et al. 2003) fail to show oestrus before the start of the breeding season. Although 10% to 30% of these cattle have ovulated but not shown oestrus, the principal problem is anovulatory anoestrus, i.e., follicular waves have restarted but the dominant follicles have undergone atresia rather than ovulation. Cows that do not show oestrus before the planned start of mating have significantly reduced fertility compared with normal cows, even if a CL is present, and it is recommended that they should be treated with a progesterone-based synchronisation programme that is timed so that synchronised cattle are inseminated on the first day of the breeding season (McDougall et al. 2005).

Monitoring Herd Fertility

In contrast to all-year-round calving herds, in which constant ongoing veterinary monitoring of fertility is a key part of the veterinarian's role, in seasonally calving farms, most fertility assessment occurs after the end of the breeding season and is aimed at identifying the issues that have occurred during that breeding season so that changes can be made in order to improve the performance in the next season.

The retrospective nature of much of the assessment lends itself to a standardised approach; a standard format for this (the fertility focus report; Fig. 26.3) has been successfully adopted in New Zealand and Australia. This report is centred on the 6-week in-calf rate (the proportion of the eligible cows that got pregnant during the first 6 weeks of mating). This is useful for benchmarking between years and between farms as it is standardised with clear criteria for numerators and denominators and with little influence of farm management choices (such as breeding season length). A cow is defined is eligible (i.e., it is included in the denominator) if she has calved on or between 130 days before and 59 days after mating start date. This calving needs to be on the farm in question, and she needs to be not culled or sold before the start of mating. To calculate the numerator only two additional pieces of information are required: the start of the mating season (which is based on the pattern of AI and is thus recorded by the insemination company) and aged pregnancy tests that confirm when during the 6-week period the cow became pregnant (recorded by the veterinarian or lay scanner). The latter requires pregnancy testing at the end of the breeding season, 11 to 14 weeks after the start of mating. Before the introduction of the fertility focus report, many herds just had a single pregnancy test approximately 6 weeks after the end of the breeding season that identified non-pregnant cows and divided pregnant ones into late (probably bull mating) and early (probably AI). So to get accurate 6-week in-calf rates has meant an additional pregnancy diagnosis. However, this has not proven a problem in most herds. In those herds that do not undertake early pregnancy diagnosis, the 6-week in-calf rate can be calculated from the percentage of the herd calved in the first 6 weeks of the calving period and the 3-week submission rate. In addition to accurate calving records (required for an accurate denominator), this calculation only requires accurate recording of insemination data rather than pregnancy diagnosis.

The requirement for accurate aged pregnancy tests (rather than just differentiating between cows pregnant in the first 6 weeks and those which became pregnant after that) is because the rates are calculated using survival analysis, with the survival curve being a key component of the fertility focus report (see Fig. 26.3). This allows the calculation of the 6-week in-calf rate to include eligible cows that have been culled or sold before the end of the period. The not-in-calf rate is calculated using the same process; this measure is very much secondary to the in-calf rate as it is strongly influenced by breeding season length, which is under management control.

The New Zealand dairy industry target of 78% was set by identifying the median in-calf rate of the top 25% of herds. A whole farm model was then used to calculate the economic effect of having a 6-week in-calf rate below that target. This showed that for a milk price of NZ$5.50 per kg milk solids (i.e., fat and protein) and a 3 cows/ha stocking density, a 1% increase in 6-week in-calf rate was equivalent to NZ$4 per cow (Brownlie et al. 2011). However, although this is a valuable calculation, particularly when identifying potential returns from improving fertility, it is only indicative. This is because two farms can have the same 6-week in-calf rate but very different reproductive performance. For example, a herd which has a 75% 6-week in-calf rate in which all the cows became pregnant on the first day of the breeding season has better performance than a herd with a 75% 6-week in-calf rate in which all the cows became pregnant on day 40. This is the same issue identified in Chapter 25 for 21-day pregnancy rate; measuring proportions over a fixed time period obscures how quickly cows get pregnant. This issue is more important for 6-week in-calf rate, than 21-day pregnancy rate as the data are accumulated over a longer period of time.

This is highlighted by the response to treatment of cows that do not show oestrus before the planned start of mating. Such treatment does not improve 6-week in-calf rate (McDougall et al. 2010); the economic benefit of synchronisation results from cows getting pregnant more quickly and calving earlier in the next season and thereby having a longer lactation (more 'days in milk'). More conventionally, synchronisation improves productivity by reducing calving-to-conception interval.

Having highlighted the 6-week in-calf rate the fertility focus report then identifies the factors underlying that performance – submission rate and conception rate. The targets for these rates are set at 90% and 60% respectively, based on obtaining a 78% 6-week in-calf rate, assuming submission rate and conception rate remain consistent over the 6-week period (this produces the dotted curve on the survival graph).

However, it is common to find changes in submission rate and, particularly, conception rate between the first 3 weeks of the breeding season and the second. For example, in Fig. 26.3 the 3-week submission rate is 93% and conception rate (based on the entire 6-week period) is 52%. Using the same calculations as in Table 26.3 would produce a 3-week in-calf rate and a 6-week in-calf rate of 48% and 73%, respectively, whereas the actual figures were 60% and 75%, respectively, consistent with a large reduction in conception rate in the second 3-week period.

The next row identifies the factors that may be underlying reproductive performance. The first column has data specifically relating to heifers – first, their calving pattern and, second, their submission rate in the first breeding season after calving. The fertility focus report excludes data from pubertal heifers as cows are only included in the denominator once they have entered the milking herd. So the calving pattern data for heifers is the first time their data is included in the fertility focus report. However, it is an extremely retrospective measure of heifer fertility as it is an assessment of a breeding season that started over 14 months

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Fertility Focus 2014: Seasonal

Report date:	27/11/17
PTPT:	
Herd Code:	
No of cows included:	248
These cows calved between:	07/06/14 and 13/12/14
Mating start & end date: (based on AB or pregnancy test data)	15/10/14 - 23/12/14
Next planned start of calving:	24/07/15
Duration of mating:	70 days
Duration of AB period:	70 days

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1 Overall herd reproductive performance

6-week in-calf rate
Percentage of cows pregnant in the first 6 weeks of mating

Your herd	83% (83-84%)
Aim above	78%

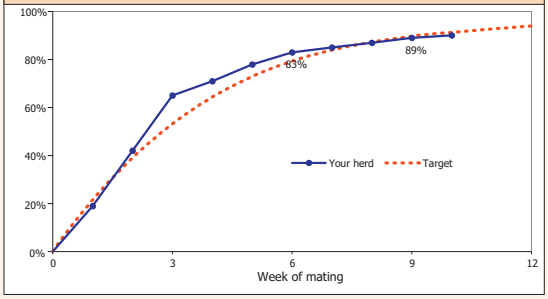
☆☆☆☆☆

Not-in-calf rate
Percentage of cows not pregnant after 70 days of mating

Your herd	10% (8-10%)
Aim for	8%

☆☆☆☆☆

% of herd in calf
Cumulative by week of mating



2 Drivers of the 6-week in-calf rate

3-week submission rate
% of cows that were inseminated in the first 3 weeks of mating

Your herd	94%
Aim above	90%

☆☆☆☆☆

Non-return rate
% of inseminations that were not followed by a return to heat

Your herd	
Aim above	

Conception rate
% of inseminations that resulted in a confirmed pregnancy

Your herd	59%
Aim above	60%

☆☆☆

3 Key indicators to areas for improvement

Calving pattern of first calvers
Well managed heifers get in calf quickly and calve early.

Calved by	Week 3	Week 6
Your herd	76%	99%
Aim above	75%	92%
	☆☆☆☆☆	☆☆☆☆☆

Calving pattern of whole herd
Did late calvers reduce in-calf rates?

Calved by	Week 3	Week 6	Week 9
Your herd	71%	92%	99%
Aim above	60%	87%	98%
	☆☆☆☆☆	☆☆☆☆☆	☆☆☆☆☆

Pre-mating heats
A high % of well managed cows will cycle before the start of mating.

Your herd	90%
Aim above	85%

☆☆☆☆☆

3-week submission rate of first calvers
Well managed heifers cycle early

Your herd	94%
Aim above	90%

☆☆☆☆☆

Heat detection
A high % of early-calved mature cows should be inseminated in the first 3 weeks of mating.

Your herd	98%
Aim above	95%

☆☆☆☆☆

Non-cycling cows
Treated non-cyclers get in calf earlier.

Treated	By MSD	Wks 1-3	Wks 4-6
Your herd	0%	0%	0%

Rating	What does it tell me?	What should I do?
☆☆☆☆☆	Top result	Ideal - keep up the good work!
☆☆☆	Above average	Getting there - focus on getting the details right.
☆	Below average	Plenty of room to improve - seek professional advice.
	No result	Not enough information provided - seek help with records.

Performance after week 6
Expected not-in-calf rate helps assess management affecting performance after week 6 (including bull management and herd nutrition).

	Not-in-calf rate
Your herd	10%
Expected	7%

Seek advice

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• **Fig. 26.3** Example 'Fertility Focus' report (© DairyNZ) showing key indicators of reproductive performance across a breeding season.

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Behind Your Detailed Fertility Focus Report

Report period: Cows calved between 07/06/14 and 13/12/14.
This was the most recent period with sufficient herd records that enabled an analysis to be completed.

Calving system: Seasonal
Your herd has been classified as seasonal calving because most calvings occurred in a single batch lasting less than 21 weeks.

Level of analysis: Detailed.
Your good record keeping means a detailed analysis was possible for your herd.

Report date:	27/11/17
PTPT:	
Herd Code:	5/4124
Calvings up to this date requested for analysis:	04/05/15
No of cows included:	248
These cows calved between:	07/06/14 and 13/12/14
Mating start & end date: (based on AB or pregnancy test data)	15/10/14 - 23/12/14

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Part A) Herd records cross check

Check that the herd records in the table are complete and correct.

2014/15	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Total
No. of calvings		68	160	25									253
No. of AB matings					222	138	26						386
No. of preg tests	2							62	184				248
No. of non-aged/late aged positive preg tests									2				2
No. of cows culled or died	13		2	1	2	2			11	27	12		70

Part B) Notes on the calculations

Use the following notes to see how your results were calculated.

1 Overall herd reproductive performance

6-week in-calf rate

Your report has been based on the mating and pregnancy test results you supplied. The ACTUAL 6 week in-calf rate is shown for your herd.

Records available for not-in-calf rate

Recorded pregnant	223
Recorded empty	19
Doubtful/recheck*	1
Culled without pregnancy test	3
No record of cull or pregnancy test	2
Cows analysed	248

*Includes cows whose most recent empty diagnosis was less than 35 days after mating end date.

2 Drivers of the 6-week in-calf rate

3-week submission rate

248 cows had calving dates in the required range and were not culled before day 21 of mating and 94% of these were submitted during the first 21 days of mating.

Non-return rate

Non-return rate is not calculated when pregnancy test results provide an accurate estimate of conception rate.

Conception rate

The conception rate was calculated for 340 AB inseminations on and between 15.10.14 and 23.12.14.

3 Key indicators to areas for improvement

Calving pattern of first calvers

67 cows with eligible calving dates were recorded as calving at less than 34 months of age. The calving pattern of first calvers was calculated from their records.

Calving pattern of whole herd

253 cows had calving dates that were eligible for this report.

3-week submission rate of first calvers

65 first calvers had calving dates in the required range and were not culled before day 21 of mating and 94% of these were submitted during the first 21 days of mating.

Heat detection

118 cows at least 4 years old at calving had calved at least 8 weeks before mating start date and were not culled before day 21 of mating and 98% of these were submitted during the first 21 days of mating.

Pre-mating heats

248 cows had calving dates in the required range and were not culled before day 21 of mating and 222 of these had a pre-mating heat recorded.

Non-cycling cows

No cows were identified as being treated for non-cycling. If you did treat non-cycling cows, please supply records to ensure those cows are identified.

Performance after week 6

Your herd's not-in-calf rate and 6-week in-calf rate were used to determine the success of your herd's mating program after the first six weeks. If bulls were used after week 6 of mating, this gives an assessment of how well they got cows in calf.

Induced cows

No cows were identified as having induced calvings. If cows were induced, ensure all inductions are recorded.

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• **Fig. 26.3, cont'd**

TABLE 26.3 Calculations underlying target conception rate (CR) (60%) and submission rate (SR) (90%) from Fertility Focus Report[a]

Number pregnant at 3 weeks	SR*CR*herd size = 0.90*0.6*100	54
Number getting pregnant between 3 and 6 weeks	SR*CR* non-pregnant cows = 0.9*0.6*46	25
Total pregnant at 6 weeks		79

[a]Assuming 100 cow herd.

ago. Any intervention resulting from this assessment is only going to influence the heifers that will be calving in two seasons time as the heifers that will be calving in the next season have already been bred. This highlights the importance of monitoring heifer weights as this is the best way of ensuring that heifer fertility is optimal. The second heifer measure, heifer 3-week submission rate, separates out first lactation heifers from the main herd because poorly grown heifers often fail to show oestrus quickly after calving for the first time.

As discussed earlier, calving pattern is crucial as late calving cows have less time to get pregnant. The fertility focus report thus provides an overall calving pattern as well as the heifer one. The next two factors, heat detection and premating heats, focus on heat detection. The heat detection measure used in the fertility focus report is an attempt to identify the proportion of false negatives (i.e., how often a cow that was on heat is missed). The program identifies a group of cows that are highly likely to show heat – cows that were at least 4 years old at calving, which had calved at least 8 weeks before mating start date and were not culled before day 21 of mating. The assumption is then made that if these cows do not show heat, this is a failure of observation not of return to cyclicity. This measure does not identify false positives; this is best done using interoestrous intervals, which are not included in the fertility focus report.

Identifying the proportion of cows that show premating heats is the best measure of identifying cows that show anovulatory anoestrus. Unfortunately getting accurate results requires the farmer to record the heats (as they are not followed by mating); on many farms, such heats are not recorded or uploaded, or if they are, they are only recorded for a short period of time.

Timing of treatment of 'non-cyclers' (cows that do not have premating heats) is important because it strongly affects the economic benefit of such synchronisation. On average, treating non-cyclers increases days in milk by approximately 14 days per treated cow (McDougall et al. 2010). Much of this effect is because synchronised cows are inseminated on day 0, whereas untreated cows, which come into heat evenly across the whole of the first 3 weeks, are, on average, inseminated on day 10. If treatment is delayed until after the start of the breeding season, the treated cows lose this advantage as they cannot be inseminated until treatment is complete, 10 days later. So, for example, if treatment starts on the first day of the breeding season, then both treated and untreated cows will be inseminated, on average, on day 10.

The final measure on the fertility focus report, performance after week 6, is an attempt to measure the performance of the bulls talking into account the 6-week in-calf rate; so for farms with a poor 6-week in-calf rate, the target not-in-calf rate is increased. As with the 6-week in-calf rate, this calculation assumes that the conception rate and submission rate remain at 60% and 90%, respectively. This is always likely to be an overestimate as it assumes that cows that have failed to get pregnant during the first 6 weeks have the same underlying fertility as cows that did get pregnant.

Autumn or Split Block Calving

Seasonal calving systems other than spring calving at pasture are relatively common in many areas of the world. Compared with pasture-based spring calving herds, autumn calving herds are generally more intensive, with the aim of achieving the group feeding benefits of a seasonal calving pattern while feeding supplementary feed (often a total mixed ration) rather than being dependent on grazing. Nevertheless, autumn calving herds often make use of spring grazing to support the later part of lactation, reducing feed cost. The principles of managing and monitoring fertility in this system are broadly similar to those in a spring calving system. However, under European conditions the increased milk yield is often associated with reduced fertility, so targets (especially for conception rate) need to be adjusted accordingly, and the expectation will be that calving period will be slightly longer than in spring. In contrast, under Australasian conditions, the fertility of autumn calving herds is often higher (and they often have shorter calving periods). This is because of the greater consistency of feed intake, both after calving (supplementary feed not just pasture) and in mid- to late lactation (spring/summer grass), which results in better body condition score.

In many farms split (or 'double') block calving systems are used. Such systems maintain the management and efficiency benefits of block calving while maintaining a relatively stable milk production through the year. Again, the key principles of monitoring and management discussed for spring calving herds apply here, although if only one set of data is available for the two herds, performance measurement can be challenging. For this reason the fertility focus system produces two reports (separated based on calving date) for block calving herds. Maintaining a sufficiently short calving period length so that there are some 'rest' times in the year can be challenging; when calving blocks are both 12 weeks long (the conventional target), there is very little of the year in which there are not cows either calving or being bred. In some cases, this leads to inappropriately short breeding periods and too many cows 'rolling over' from the autumn to the spring calving herd or vice versa, which is likely to have a substantial effect on profitability.

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27

Veterinary Control of Reproduction in Beef Herds

NEIL SARGISON AND COLIN PENNY

Basic Economics and Targets of Suckler Beef Calf Production

Suckler beef cattle fulfill a role in food production from marginal agricultural settings that are poorly suited to arable farming and cereal-based livestock production. The economic stability of systems of suckler beef production is driven by the efficiency of herbage growth and optimisation of its conversion into beef meat. At an animal level, this equates primarily to 'weaning the optimal total weight of calves per unit of cows'. Differential calf market values per kilo, depending on conformation and integration with other farm management, are secondary considerations. These production objectives differ greatly from intensive dairy production (see Chapter 25) in which there is a strong emphasis on efficiency of supplementary feeding to meet the nutritional demands of milk production, but there are many similarities to pastoral dairy farming.

Cattle must be cared for over the winter months when herbage availability and quality in marginal areas is poor. This incurs high costs of housing, labour, machinery, conserved forage, supplementary concentrate feeds, and disease management (Fig. 27.1). Therefore, in the absence of differential value chains, the most economically efficient systems are those that minimise these costs by matching the nutritional requirements of the suckler beef cattle production cycle to the seasonal availability of and access to herbage. The peak metabolic requirements of suckler cows arise during lactation, whereas the lowest needs are after weaning. These can be balanced by the cow to some extent through mobilisation of body reserves during late pregnancy. Hence calving in the spring coordinates lactation with herbage growth and availability, and allows cows to accumulate body reserves to tide them over late pregnancy. This allows calves to be weaned in the autumn or early winter at 6 to 8 months old, matching the dry cows' more modest nutritional requirements with poorer herbage availability and quality. Spring calving is amenable to precise management of nutrition, housing, and disease during the second half of pregnancy. Conversely, autumn calving is expensive in terms of feed management and the need for housing and incurs greater costs of disease prevention in growing calves because precise nutritional management is problematic during later stages of pregnancy on pasture. Nevertheless, autumn calving does allow utilisation of conserved forage and can complement general mixed farm management in some circumstances. This chapter will focus on spring calving, suckler beef cattle herds (Fig. 27.2) as an efficient model system that is suited to upland western European settings.

Historic 'headage-based' subsidy payments and traditional small scale mixed farming systems encouraged the achievement of a 100% pregnancy rate as the primary reproductive management target for beef suckler cow herds. Provided that periparturient disease and infectious causes of infertility such as venereal campylobacteriosis were absent, this target can be achieved by running sound bulls with the herd all year round. However, such management inevitably resulted in mean calving intervals of individual cows greatly exceeding 365 days, and consequently, they will be calving all year round. Failure to match herbage availability to the cows' metabolic requirements created a need for longer housing periods and greater labour inputs to meet the complicated nutritional management needs of multiple small groups of individual animals. The greatest economic effect resulted from calves having mixed ages and weights at fixed weaning times and sale dates, with the mean weight being lower than if the calving period had been compact. Although the system worked for small herds finishing their own calves in small batches, it is not conducive to efficient management of large groups of animals with economics of scale. A compact calving period is therefore necessary both to optimise the utilisation of herbage growth and to ensure the maximum total weight of calves at weaning on a fixed date. A mean calving interval of 365 days is a prerequisite to maintain this year-on-year.

The traditional model of suckler cow beef production encouraged added value of individual calves through better conformation. Throughout the 1970s and 1980s, typically small Hereford, Aberdeen Angus, or Shorthorn-cross dairy cows were replaced by larger beef bred animals, and traditional purebred early maturing bulls were replaced by larger, late maturing Continental European breeds, in particular the Charolais, Simmental, Limousin, and Blonde D'Aquitaine, among various other breeds. Early maturing breeds were well suited to extensive, pastoral production systems, whereas later maturing animals required intensive management to allow them to grow quickly and to achieve target weights for their age. When managed accordingly, the late maturing cattle had higher slaughter weights, higher carcass weights, better carcass conformation, and lower carcass fat scores than early maturing animals slaughtered at similar ages. However, this was achieved at the expense of a high reproductive cost of dystocia and associated consequences of extended postpartum anoestrous periods, failure to rebreed, and increased calf mortality (Fig. 27.3). Today,

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• **Fig. 27.1** The necessity to house beef suckler cattle over winter incurs high costs and creates opportunities for the spread of disease between cows and their calves.

• **Fig. 27.2** Spring calving is suited to the conversion of herbage into calf growth and affords an efficient model system for reproductive management.

• **Fig. 27.3** Severe dystocia risks death of the newborn calf and of its dam, whereas the consequences of moderate birth stress include poor calf growth and a prolonged postpartum anoestrous period. At a herd level, this reduces the value of calves weaned, extends the subsequent calving period, and, if the mating period is compact, increases the barren rate.

• **Fig. 27.4** A spring calved Angus crossbred cow with an easy calving Limousin sired calf.

TABLE 27.1 Typical basic targets for spring calving beef suckler herds

Key Performance Indicator	Target
Barren cow rate	< 5%
Mature cows with difficult calvings	< 5%
Calf mortality (birth to weaning)	< 3%
Cows/heifers calved in first 3 weeks	≥ 65%
Calves weaned per cows/heifers mated	≥ 94%
Mating period for adult cows	9 weeks
Mating period for heifers	6 weeks
Calving period	< 10 weeks
Replacement rate	< 15%

compromises have been reached, in part driven by a move away from dairy-bred beef cows, which became unattractive due to an increasing Holstein influence. However, these still typically involve relatively large cows, commonly Angus or Limousin crossbreeds, with mature weights of 500 to 700 kg, coupled to selection of bulls for easier calving of their offspring (Fig. 27.4). These characteristics affect the reproductive management that is required to achieve a compact mating period and 365–day calving interval.

Some typical targets for UK and Irish spring calving beef suckler herds are shown in Table 27.1. When benchmarking beef suckler herd fertility performance, the two most useful key performance indicators (KPIs) are the weaned calf crop, expressed as a percentage of cows mated, and cows calved in the first 21 days, expressed as a percentage of all cows calving. The weaned calf crop is helpful as it factors in losses from the whole production cycle from conception through pregnancy loss to calf mortality. The percentage of cows calving in the first 21 days reflects both cow and bull fertility, and is an index of general cow reproductive and nutritional management and bull stud breeding soundness. Benchmark figures need to be calculated correctly to ensure comparable information. A standard gestation period for the farm, commonly 285 days, is added to the start date for mating to calculate the theoretical starting date for the calving period; then all cows calved in the 3 weeks after this date are counted, plus any cows calving before the calculated calving start date. Counting 3 weeks from the date, the first cow calves can reduce the benchmark figures and reduce the value of between-herd comparisons.

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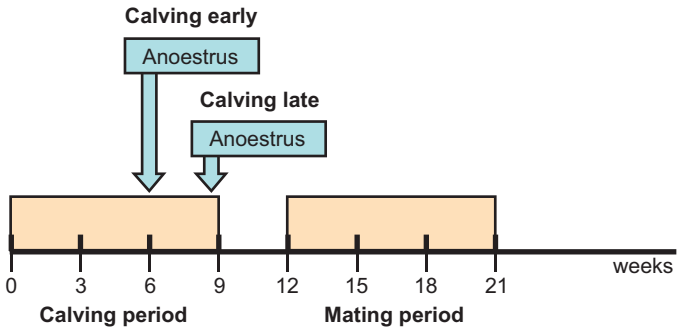


• **Fig. 27.5** The effect of stage of calving of an individual cow within the herd calving period on rebreeding opportunities after a 42-day postpartum anoestrous period.

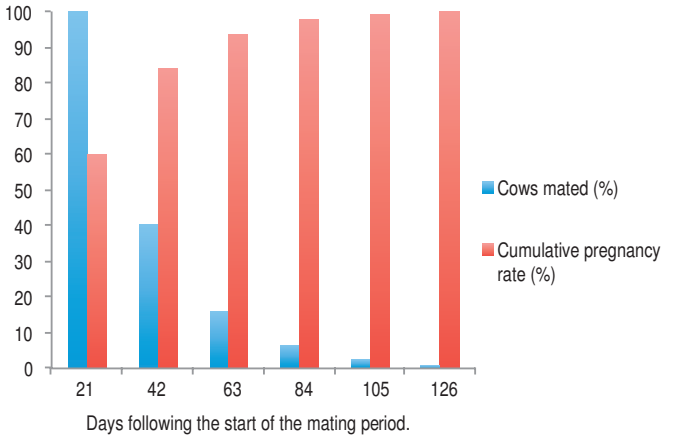
Although some UK and Irish beef farmers achieve the fertility and production goals shown in Table 27.1, many fall short. Actual benchmarking data shows average figures for cows calved in first 3 weeks of less than 50% and for weaned calf crops of 85% or less are commonplace. This demonstrates the scope for increasing fertility and production efficiency in beef suckler herds.

Theory of Beef Cattle Reproduction

The main annual reproductive target for spring calving suckler beef herds is to wean the maximum value of calves per hectare. This is achieved primarily by optimising the number of calves produced per group of cows while maximising the mean age, and therefore weight, of calves on a fixed weaning date. Maximisation of the value per kilogram of weaned calves is a secondary consideration to achieving ease of calving and rebreeding, but can be greatly influenced by selection of bulls with superior estimated breeding values (EBVs) for calving ease and growth.

The time interval between conception and calving in beef cows is between about 275 and 290 days (commonly assumed 285 days), generally being shorter for smaller, early maturing native breeds and longer for larger, late maturing Continental breeds. Beef cows that are in good body condition and calve easily without assistance have a postpartum anovulatory anoestrous period of between about 30 and 35 days; hence after a first silent ovulation, cows typically do not show first behavioural oestrus until 40 to 50 days postpartum (Crowe et al. 2014). In order to maintain a 365-day calving interval, bulls must be introduced about 80 days (365 minus 285 days) after the planned start of calving date. Hence with a target mating period of 63 days (three oestrous cycles) (Mossman & Hanly 1977), it is clear that, even in ideal conditions in which all cows have resumed oestrous cycles by 42 days postcalving, the later calved cows are unlikely to be cyclical at the start of the mating period. However, by calving the majority of cows in the first 6 weeks of the calving period, there will be sufficient recovery time to ensure most cows have up to three mating opportunities within the restricted mating period as shown in Fig. 27.5.

Economically efficient spring calving beef suckler herds all have a compact calving period, whereas most problem herds have calving periods longer than 12 weeks, not necessarily associated with low pregnancy rates due to extended mating periods. Maintenance of a compact calving period has additional benefits. Supervision of calving and calf disease management is easier because opportunities for the environmental build-up of pathogens are lower. Feed budgeting is more efficient for groups of late pregnant cows with known calving dates spread over a short time period.



• **Fig. 27.6** Theoretical pattern of mating and pregnancy over a six-oestrous cycle period.

Provided that heifers or cows are cycling and the bulls are reproductively sound or that artificial mating methods are effective, the average pregnancy rate after each behavioural oestrous event is around 60% (Mossman & Hanly 1977). Theoretical pregnancy rates resulting from mating periods spanning multiples of 21-day oestrous cycles can therefore be predicted at a herd level (Fig. 27.6). This theoretical model is consistently validated in practice (Hanly & Mossman 1977, Diskin & Kenny 2014).

This theoretical model shows that about 94% of the cows will be pregnant by the end of the 63-day mating period. Achievement of a compact calving interval using natural mating therefore potentially incurs a 6% barren rate. The model assumes that all of the cows were cycling when the bulls were introduced, in which case a 105-day mating period would be required for nearly 100% to become pregnant. However, in this scenario, 6% of the cows will not be calved, or cycling, when the bulls are introduced during the next year, thus increasing the length of the mating period required to achieve a 100% pregnancy rate, year-on-year. The subsequent extended calving period will result in poor weaning weights of later born calves and a lower average calf value at weaning on a fixed date. The extended calving period will allow for the build-up of environmental pathogens, increasing the requirements for disease management because nutritional management of cows calving over an extended period will be inefficient.

The fact that some very well managed beef suckler herds with compact 63-day mating periods can achieve pregnancy rates of

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95% or greater, even when it is biologically unlikely that 100% of cows are cyclical on Day 1 of mating, shows that conception rates to first mating in beef cows may potentially be 70% or higher.

Practical Management of a Compact Mating Period

The achievement of a 94% pregnancy rate after a 63-day mating period is dependent on the majority of heifers and cows cycling at the start of the mating period. Heifer management, cow nutrition and avoidance of postparturient disease are critical in this regard.

The onset of puberty in heifers is controlled by body weight ahead of age. Although there are differences between breeds and management systems, more than 90% of *Bos taurus* heifers will have displayed behavioural oestrus once they have reached 60% to 65% of their mature body weight (see Chapters 3 and 25). Fertility to the first pubertal oestrus is lower than to subsequent oestrous events (Byerley et al. 1987); hence puberty should ideally be attained at least 1 month before commencing mating. Furthermore, heifers that are mated and conceive before reaching a critical minimum weight will calve at lower body condition scores (BCS) and lighter weights than their contemporaries, thus with a high risk of dystocia and failure to rebreed due to prolonged postpartum anoestrus. Strategies to hasten onset of puberty in beef heifers include treatment with progestogens or progesterone products and exposure to vasectomised bulls, but neither option is commonly adopted in European beef herds and the response may be inconsistent (Diskin & Kenny 2014).

In spring calving herds, which source breeding replacements from homebred animals, or from other spring calving herds, practicalities dictate that heifers must calf for the first time at either 2 or 3 years old. Calving at 3 years old incurs higher maintenance costs of unproductive animals than calving at 2 years old and also reduces the potential productive lifespan and output of the female breeding herd. Conversely, calving at 2 years old provides a good basis for selecting replacement animals and potential for faster genetic gain. Hence most producers now aim to calve their heifers for the first time at 2 years old. This means that they must reach 65% of their mature weight by a mean age of 15 months. This equates to critical minimum mating weights of between 325 kg and 420 kg for small Angus and large Continental European bred animals, depending on the characteristics of their breed; this requires mean live weight gains of between 0.65 kg/day and 0.84 kg/day from birth. Achieving these targets is relatively straightforward for small early maturing animals but depends on high levels of supplementary feeding as part of a balanced ration for late maturing breeds. Overfeeding of early maturing breeds in particular, in an attempt to achieve critical minimum mating weights, compromises behavioural oestrus, conception rates, embryonic survival, and mammary gland development in fat animals, and increases the risk of dystocia and postparturient infections. Control of infectious diseases, such as bovine respiratory disease, roundworms, and liver fluke, in growing heifers is critical to ensure growth targets are achieved.

Failure to reach critical minimum weights at first mating of heifers results in high barren rates, whereas those that become pregnant calve at low weights and in poor body condition, with high levels of dystocia and postparturient disease. Subsequent barren rates of first calvers are high due to extended postpartum anoestrus in thin animals, resulting in less than three behavioural oestrous events during a compact three-cycle mating period.

The postpartum anoestrous period after first calving in heifers is about 20 days longer than that of cows in the same body condition. This means that if the heifers' three-cycle mating period is the same as that of the cows, then during the subsequent year, first calvers will only have two opportunities to show behavioural oestrus; therefore, on average, 16% will remain barren. Furthermore, those that become pregnant will calve correspondingly later and remain late calving cows for the remainder of their reproductive lifespan, with a higher risk of not becoming pregnant before the end of the mating period than in cows that calve early. Their calves that are born late in the calving period will be younger and lighter than those born earlier and therefore of lower sale value. In herds in which heifers are first calved at 3 years old, this problem is addressed by advancing the heifer mating period by 3 weeks compared with that of the main cow herd. However, in herds in which heifers are first calved at 2 years old, this strategy risks more animals failing to reach their critical weights at mating. A two-cycle (6 weeks) heifer mating period, starting at the same time as the three-cycle mating period for the main herd, offers a satisfactory compromise, allowing all of the heifers that calve to be cycling at the start of the subsequent mating period. The compromise involves mating more heifers than are required, as perhaps only 84% will conceive, but potentially 71.5% (60% x 100/84) of those that become pregnant will calve during the first 3 weeks of the calving period. In practice, if heifers have all reached their critical mating weights and are run with fully sound bulls, it is not unusual to achieve pregnancy rates of greater than 90% in a 6-week heifer mating period. The opportunities for alternative economically efficient management of less than 2-year-old barren maiden heifers are greater than those for older barren first or second calvers.

Heifer management dictates the reproductive performance of the whole herd and is key to achieving reproductive targets. Heifers need to be fed for maintenance plus 0.65 kg/day to 0.85 kg/day growth to ensure that they achieve critical minimum weights and are in good, but not excessively fat, body condition at mating. Heifers must be mated with bulls that have been selected for ease of calving. The same plane of nutrition must be maintained after mating to ensure that heifers calve in body condition of 3.0 on a scale of 1 (very thin) to 5 (very fat), based primarily on palpation of the transverse processes of the lumbar vertebrae. This is relatively straightforward for spring calving heifers at pasture (Diskin & Kenny 2014) but requires a carefully balanced winter feeding ration in autumn calving herds. Higher BCS predisposes to dystocia, whereas lower scores result in extended postpartum anoestrous periods. After calving, heifers that were first mated at 15 months old benefit from separate, beneficial management until their second calving at 3 years old. The differential disease and nutritional management of heifers and first calvers needs to be planned and affords opportunities for veterinary involvement in achieving beef suckler cow reproductive targets. Prebreeding scoring of heifer reproductive tracts by palpation or transrectal ultrasonography of the uterus and ovaries can be helpful to identify individual animals with abnormal tracts, e.g., freemartins (see Chapter 9) and those that that are prepubertal and therefore likely to fail to become pregnant in a restricted mating period (Holm et al. 2015).

The postpartum anoestrous period of mature cows is determined by the nutritional demands of the previous pregnancy and lactation and is related to BCS at calving, plane of nutrition, and the calf suckling and maternal bond. Temporary calf isolation or restricted suckling from around 30 days postpartum may significantly

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• **Fig. 27.7** An Angus cow at the beginning of the mating period in June with BCS 3.0 (1-5 scale).

• **Fig. 27.8** It is recommended that all beef bulls should be checked for breeding soundness before the start of the mating period.

shorten the time to first postpartum ovulation of beef cows (Stagg et al. 1998) but is impractical in most spring calving herds on pasture.

Cows calving in good BCS have the shortest postpartum anovulatory anoestrus. Low body condition (i.e., < 2.5 on a 5-point scale) leads to proportionally increased calving to conception intervals and later subsequent calving dates or to high barren rates. Increasing levels of feeding after calving has little beneficial effect in cows that calve below target BCS; hence it is important to manage pasture, forage, and supplementary feeding throughout the year based on monitoring of BCSs and comparison with target values.

In order to minimise the duration of postpartum anoestrus, the target BCS at calving is between 2.5 and 3.5 on a 5-point scale. For spring calving beef suckler herds, in which the objective is to maximise the utilisation of herbage growth during lactation, this dictates a minimum target BCS of 2.0 at mating and 3.0 at weaning (Fig. 27.7). For autumn calving beef suckler herds, which depend on expensive forage and concentrate feeding during the winter to meet the demands of lactation, minimum BCS targets of 3.0 at calving, 2.5 at mating, and 1.5 at turnout in the spring are appropriate, depending on the precise nature of the system (Lowman 1988).

In practice, a relatively inexpensive ration of ad lib straw and 2 kg of concentrate feed is sufficient for maintenance of a pregnant spring calving cow over winter, provided that she enters winter in the correct body condition. Management of herbage height controls nutrition during the summer but is less predictable than housed rations, commonly resulting in autumn calving cows being too fat at calving.

Pregnancy diagnosis and determination of fetal age by transrectal ultrasonography is useful in herds with a compact mating period to confirm that reproductive targets are met and instigate timely investigation if they are not. Good farm records of reproductive management are important in this regard, including: numbers of cows or heifers put to the bull; number of bulls used; dates that bulls were introduced and removed; number of cows or heifers diagnosed as pregnant; numbers of calves born alive; first and last calving dates; number of cows or heifers calving in each 3-week period; and details of animal deaths or culling. Pregnancy diagnosis allows barren animals to be identified early and informed decisions to be made concerning what to do with them. This can be particularly helpful in the case of heifers, allowing nonpregnant animals to be removed and economically managed for finishing. Simple analysis and benchmarking of KPIs can be undertaken using the same data discussed previously and allows ongoing monitoring of herd performance.

Other Factors Affecting Beef Suckler Herd Reproductive Performance

The basic reproductive management of spring calving beef suckler herds with a compact mating period first depends on appropriate heifer management and maintaining target BCS in cows. Another critical factor is bull fertility and, in individual herds, infectious diseases and specific nutritional deficiencies can also affect the achievement of economic reproductive targets.

Clearly, to achieve and maintain reproductive targets in beef suckler cattle, it is crucial that the herd bull stud is fully fertile and capable of delivering conception rates of 60 to 70% to each service throughout the mating period (Fig. 27.8). In many herds, the presence of subfertile and unsound bulls due to factors such as lameness and poor semen quality will contribute to reproductive failure. Published surveys have consistently found that in an unselected population of beef stock bulls, the failure rate when subjected to a full breeding soundness examination can be 20% or higher. It is therefore important that all bulls are subjected to a breeding soundness examination before each mating period (see Chapter 35). Healthy, mature bulls that have passed a breeding soundness examination can be run with breeding groups of 30 to 40 cows, whereas younger bulls must be limited in their first breeding period to groups of 15 to 20 females. Consideration also needs to be given to the terrain and size of fields used to accommodate the breeding group during the mating period of spring calving herds, e.g., avoiding steep slopes and large enclosures. It is common practice to rotate bulls around breeding groups halfway through the mating period to help limit the effect of possible bull subfertility or infertility; however, farmers should have confidence to run a single sire with mating groups if they have had a prebreeding examination. Introduction of bulls that have previously been used for breeding, especially hire bulls, should be avoided as it can have severe biosecurity implications, risking introduction of venereal diseases such as campylobacteriosis and trichomonosis.

Generalised infectious diseases caused by bovine viral diarrhoea pestivirus (bovine viral diarrhoea – BVDV) or bovine herpes virus-1

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(infectious bovine rhinotracheitis – IBR) can result in failure of conception or maintenance of pregnancy. Specific pathogens, such as *Salmonella* Dublin and other serovars, *Leptospirahardjo,* and variants of *Campylobacter fetus* subsp. *venerealis,* cause reproductive loss in European beef suckler herds through abortion or embryonic losses. Introduction of these diseases to naïve herds can have a large effect on reproductive performance, and their presence in endemically infected herds can compound reproductive losses due to other causes. The pathogenesis of these diseases is described in Chapter 24. The control of BVDV, BoHV-1, salmonellosis, and leptospirosis in endemically infected suckler herds can involve eradication and vaccination. Nonspecific pathogens, such as *Listeria monocytogenes* and *Bacillus licheniformis,* cause sporadic abortion losses as a consequence of feeding of spoilt silage. In beef suckler herds, *Neospora caninum* is generally only diagnosed as a cause of abortion in association with other infectious diseases or poor nutrition.

Venereal campylobacteriosis has been shown to be economically important when identified in beef suckler herds (Truyers et al. 2014), causing embryonic mortality and occasional abortions. However, without good farm records as a stimulus for the investigation of poor reproductive performance and availability of practical sensitive and specific diagnostic tests, the true prevalence of this infection is unknown. It is possible that a high prevalence of venereal campylobacteriosis may be responsible for failure to reach reproductive targets in many beef suckler herds. Artificial insemination has been proposed as a simple control method for bovine venereal campylobacteriosis, but it is impractical for most spring calving beef suckler herds when breeding takes place at pasture. Commercial vaccines are unavailable in Europe but are widely used in other continents, and the efficacy of autogenous vaccines using a bacterial isolate from infected animals on a specific farm is at best unproven. For most infected herds, control requires good record keeping allowing biocontainment of the disease by segregation of potentially infected and uninfected animals in combination with culling or treatment.

Prevention of infectious causes of poor reproductive performance in naïve beef suckler herds depends on good biosecurity in conjunction with good general farm management. Most beef suckler herds are comprised of crossbred cows mated to purebred terminal sire bulls. In these situations, maintenance of totally closed, and therefore biosecure, herds is impractical without artificial control of reproduction; consequently, most beef suckler herds regularly introduce new bulls. Many herds also buy in replacement crossbred breeding heifers. Trade in calved heifers and cows is commonplace but is strongly discouraged due to the disproportionately high risk of introduction of generalised or specific infectious diseases, such as BVDV and BoHV-1 or venereal campylobacteriosis, respectively. Attempts must be made to purchase replacement heifers or bulls from herds with similar disease status, albeit this is often unknown. Introduction of animals to bovine tuberculosis-free areas from endemically infected regions is strongly discouraged. Purchased animals need to be quarantined on arrival for sufficient time – usually 1 month – to allow them to be tested for specific diseases, vaccinated against those infectious diseases that are endemic in the new herd, or show clinical signs caused by infections that were latent on arrival.

Deficiencies in copper, selenium, and iodine are frequently implicated as causes of poor reproductive performance in pastorally managed beef suckler herds, but in many cases, these problems arise secondary to poor basic nutritional management. The pathogenesis and management of trace element deficiencies is in Chapter 22.

Artificial Breeding Management of Beef Suckler Herds

Artificial insemination (AI) has been used for many years in pedigree beef breeding herds along with other breeding technologies, such as multiple ovulation embryo transfer; but current estimates from US and Europe suggest that only around 10% of commercial suckler beef cattle are bred by AI. Economic arguments for AI in commercial beef herds revolve around the ability to inseminate large numbers of cows or heifers on Day 1 of the mating period and to utilise superior sire genetics suited to the production system. Assuming acceptable pregnancy rates to AI (target 60%–65%), this can lead to economic gain by having a high percentage of the herd (typically > 70%) calved in 21 days, resulting in an increased mean value of weaned calves from a combination of extra age growth and superior genetics. A well-managed, fixed-time AI (FTAI) programme in beef cows will consistently deliver a superior calving pattern to a natural service alone as it takes at least 21 days for every female to be exposed to the bull, as opposed to service on 1 day by FTAI.

Field studies have demonstrated that by using oestrous synchronisation and FTAI for first service followed by sweeper bulls, beef cows can have superior reproductive performance and economic gain compared with using natural mating alone (Rodgers et al. 2012). Another common reason for using AI in beef cows or heifers is to access sires with suitable maternal trait EBVs, helping to breed superior replacement heifers. Keeping heifer calves that have been bred from terminal sire stock bulls as replacements may be unwise as it can lead to an unsuitable cow type with poor milk production. The growing availability of sexed semen opens up further opportunities to breed heifers from heifers or increase the proportion of male calves born.

The ability to synchronise oestrus, leading to single FTAI with minimal labour inputs and handing protocols, is crucial. Many protocols have been described that allow synchronisation of the oestrous cycle in beef cows and heifers for FTAI involving use of prostaglandin $F_{2\alpha}$ (PG) and gonadotrophin releasing hormone (GnRH) injections, intravaginal progesterone implants and oral progestins (see Chapter 8). In general, the use of double PG injection or GnRH/PG/GnRH protocols is less successful than reported in dairy cattle, because a proportion of animals will be in anovulatory anoestrus in beef suckler herds. Progesterone-based programmes are therefore the method of choice, as progesterone treatment alone or in combination with 400 to 500 IU equine chorionic gonadotrophin (eCG) has been shown to overcome anovulatory anoestrus in beef cows and lead to a normally fertile oestrus in most cases (Patterson 2016). This allows cows to be synchronised and bred by as early as 40 days postpartum, and achieve pregnancy much earlier than had they been exposed to bulls alone. Reproductive tract scoring of groups of heifers to ensure that they are all cyclic before synchronisation can further improve pregnancy rates after FTAI. When sweeper bulls are introduced to cover repeat services, sufficient bull power must be employed to allow for synchronisation over a period of about 7 days (Fig. 27.9). Repeated oestrus synchronisation and AI protocols have been described, allowing suckler beef cows to be bred entirely without the need for bulls (Penny et al. 1997), but as these involve multiple handlings, they are considered by some to be impractical.

For protocols utilising intravaginal progesterone implants, the decision on which to use is often guided by previous experience and the number of handlings that are considered acceptable. It is

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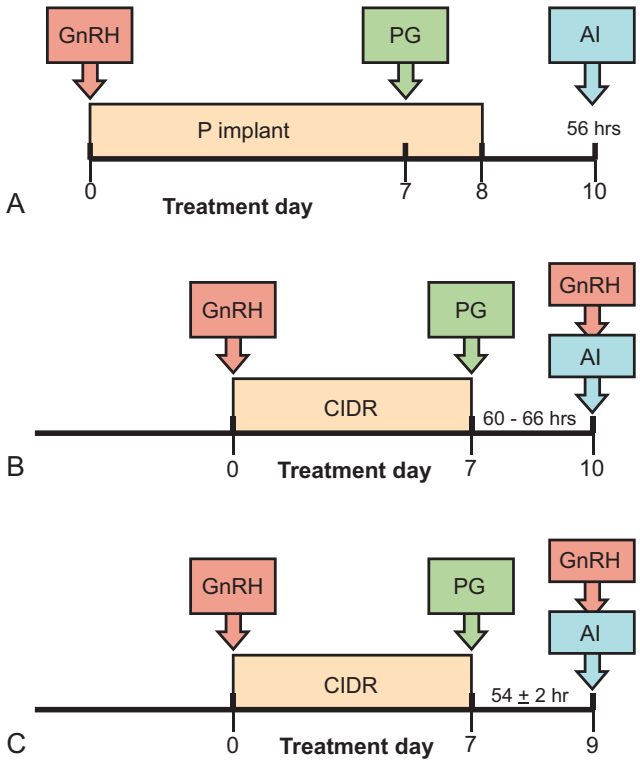
• **Fig. 27.9** Sweeper bull power (both breeding soundness and numbers) needs to be sufficient to take into account that, if not pregnant to their first service or mating, heifers or cows synchronised for their first cycle will all return to a second behavioural oestrus within a short time period.

noteworthy that, even using identical protocols, the between-farm variation in results can be large, showing the importance of other management factors in success. Most product data sheets suggest PG injection at least 24 hours before progesterone implant removal, which ensures that any existing luteal tissue is already lysed, thus tightening synchrony. In the US, it is more normal to use 'Co-Synch' protocols (Patterson 2016) in which PG is administered on the same day as progesterone implant removal, thus reducing a handling. Examples of typical progesterone implant based protocols for beef cows or heifers are shown in Fig. 27.10. Administration of GnRH injection at progesterone implant insertion may be beneficial as it reduces the risk of dominant follicle persistence and starts a fresh follicular wave in the majority of females.

Consistent results can be achieved with FTAI in beef cows and heifers by:

i) Ensuring good forward planning and compliance with the protocol;
ii) Ensuring that cows and heifers are in suitable BCS and target mating weights so that majority are cyclic at start of programme;
iii) Having suitable handling facilities and avoiding stressful management such as administration of vaccines close to the AI date;
iv) Maintaining a steady or rising plane of balanced nutrition;
v) Controlling infectious diseases and nutritional deficiencies that could affect fertility; and
vi) Using skilled technicians and quality semen.



• **Fig. 27.10** Progesterone implant based protocols for fixed time artificial insemination in beef cows or heifers. P: progesterone. CIDR: controlled internal drug release (progesterone). GnRH: gonadotrophin releasing hormone. PG: prostaglandin. AI: artificial insemination. (A) Typical UK progesterone based protocol for beef suckler cows or heifers. (B) Minimal handling 'Co-Synch' protocol for cows. (Source: Applied Reproductive Strategies in Beef Cattle (ARSBC), Estrus Synchronisation Protocols for HEIFERS and COWS, https://beefrepro.unl.edu/resources.html, accessed 13 March 2018). (C) Minimal handling 'Co-Synch' protocol for heifers. (Source: ARSBC, Estrus Synchronisation Protocols for HEIFERS and COWS, https://beefrepro.unl.edu/resources.html, accessed 13 March 2018.)

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Fertility and Infertility in *Bos indicus*

ROBERTO SARTORI, JÉSSICA NORA DRUM AND ALEXANDRE BARBIERI PRATA

Introduction

Over the years, cattle have been raised and selected especially for meat and dairy production; the majority of breeds are either *Bos taurus* (*B. taurus*) or *Bos indicus* (*B. indicus;* Zebu), see Fig. 28.1. Some of the *B. taurus* breeds, such as Holstein and Jersey, have been selected for high milk production, and others, such as Angus and Hereford, for beef production. Due to the intensive selection, *B. taurus* breeds are very efficient for food production compared with other breeds; however, they are not adapted to tropical environments and cannot make use of their entire potential for production in the tropics. The main problem with the husbandry of *B. taurus* in the tropics is their very low tolerance to heat stress; compared with *B. taurus,* Zebu breeds experience a less severe reduction in feed intake, growth rate, milk yield, and reproductive function in response to heat stress (Adeyemo et al. 1979, Bennett et al. 1985, Pegorer et al. 2007, Satrapa et al. 2011).

Although *B. taurus* breeds are more precocious, Zebu cattle are extensively used in the tropics, due to their thermo tolerance and resistance to parasites. The extensive systems of management, which predominate in South America, have continually exposed them to a tropical climate and nutritional environment, resulting in increased tolerance to that environment through selection pressure. This environmental tolerance means that Zebu cattle can thrive, whereas *B. taurus* animals may not. However, regarding reproductive management, difficulties of oestrous detection in Zebu cattle is one of the major factors that limit the widespread use of AI in these breeds. This can be overcome with the use of fixed-time artificial insemination (FTAI) protocols, developed or adapted specifically for Zebu cattle. Additionally, embryo production and transfer has been increasing around the world, especially in tropical countries such as Brazil, particularly because of the use of *in vitro* production in Zebu breeds or crossbreeds (Viana & Camargo 2007, Viana et al. 2017).

To overcome or minimise problems related to decreased production of pure bred *B. taurus* cattle in tropical regions, one strategy has been to produce *B. taurus* x *B. indicus* crossbreeds. Zebu cattle are known to be less fertile and have lower levels of milk production than *B. taurus* breeds, but their better adaptation to the environmental conditions make them more likely to reproduce successfully in the tropics. Crossbreeds incorporate the environmental adaptation of Zebu cattle, with the higher production of *B. Taurus* (Negussie et al. 1999), as well as the benefit of hybrid vigour.

Because most of the reproductive physiology studies in cattle have involved *B. taurus* breeds in countries with a temperate climate, strategies for reproductive management that have been used in Zebu cattle have been adapted from those studies and may not be ideal for *B. indicus.* Although the majority of studies reported in the literature have used *B. taurus* cattle to study ovarian physiology, there are an increasing number of reports involving *B. indicus* breeds, such as Brahman and Nelore. These recent studies have gained a great deal of knowledge on the reproductive physiology of *B. indicus,* enabling direct comparisons with *B. taurus* under similar environmental, nutritional, and management conditions to be made. These studies have identified considerable differences in ovarian morphology and function, as well as circulating hormones and metabolites between these two genetic groups.

Currently, researchers are using this acquired knowledge to optimise protocols for FTAI and production of *in vivo*-derived (IVD) embryos by multiple ovulations or by *in vitro* embryo production (IVP). Accordingly, this chapter is divided into sections that describe: 1) physiological differences in reproductive cycles of *B. taurus* and *B. indicus;* 2) nutritional and metabolic effects on reproductive physiological parameters and performance; and 3) assisted reproductive technologies (ARTs) applicable to *B. indicus.*

Physiological Differences in the Oestrous Cycle of *B. taurus* and *B. indicus*

Puberty

Puberty is defined by the animal's ability to reproduce as a result of the development of hypothalamic neurones, which secrete GnRH at the appropriate frequency and in sufficient quantities to release gonadotropins and promote gametogenesis (see Chapter 3). Although the first ovulatory oestrus is the main marker of puberty (Rawlings et al. 2003), heifers usually only reach maximum fertility at their third oestrus (Byerley et al. 1987).

Increased LH pulse frequency in the circulation, which is the determining factor for sexual maturity of heifers, is due to decreased hypothalamic sensitivity to the negative feedback of oestradiol (E2; Day et al. 1987), which is influenced by genetic and environmental factors (Martin et al. 1992, Nogueira 2004).

Most heifers reared in the tropics have access to low quality forages and suffer from protein, energy, and/or mineral deficiency. Therefore they do not reach puberty until 2 years old, whereas those in a temperate climate reach puberty around 9 to 12 months of age (Fajersson et al. 1991). Independent of nutrition, there also seems to be a genetic factor associated with puberty in Zebu cattle because 30% of Nelore heifers (12 of 37) became pregnant at 16 months of age but with no difference in age or weight from

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• **Fig. 28.1** Zebu Cattle. (Courtesy of Roberto Satori).

the group mean at first ovulation (Nogueira 2004). In addition, when comparing precocious with nonprecocious sires, daughters of nonprecocious sires at puberty onset are heavier and older than precocious heifers (Ferraz et al. 2014).

There are clear differences in age at puberty between *B. taurus* and *B. indicus* cattle. Generally, Zebu heifers are less precocious than *B. taurus* heifers. This was elegantly demonstrated in the study by Rodrigues et al. (2002), in which the cessation of inhibition of E2 on LH pulses occurred simultaneously with the onset of puberty in heifers of both genotypes but occurred earlier in *B. taurus* heifers, especially during the initial period of weight gain (16–18 months of age). The estimated age at puberty for Zebu in the tropics and subtropics is between 16 and 40 months, with a mean of 25 months, i.e., 6 to 12 months later than *B. taurus* (Abeygunawardena & Dematawewa 2004, Nogueira 2004). Moreover, smaller breeds such as the Zebu normally reach puberty at an older age and heavier body weight than *B. taurus* heifers (Martin et al. 1992, Bagley 1993). Finally, regardless of size and nutrition status, Zebu cattle are less precocious because the selection pressure for early onset of puberty has been much less intensive than in *B. taurus* cattle (Eler et al. 2002), even though there is a high correlation between scrotal circumference in *B. indicus* bulls and young age of puberty in their heifer offspring (Forni & Albuquerque 2005, Eler et al. 2006). Moreover, age at first conception was highly heritable (h2 = 0.44–0.67; Pereira et al. 2006) as was heifer pregnancy (h2 = 0.58–0.66; Eler et al. 2006). There was also a high genetic correlation between age at first calving and the interval from the onset of the breeding season to calving in the subsequent year (Forni & Albuquerque 2005). Therefore the potential to advance the age of puberty and shorten the postpartum anoestrous interval through selection in *B. indicus* cattle exists.

Oestrous Cycle Overview: Follicular Wave Patterns and Ovulation

After puberty the female bovid has recurrent oestrous cycles that continue throughout most of their productive life unless she becomes pregnant or undergoes a period of negative energy balance that would induce anoestrus (Diskin et al. 2003). One of the first studies performed in Nelore cattle reported that oestrous cycle length was on average 20.7 (n = 20) and 22.0 (n = 14) days for two and three follicular wave animals (see Chapter 1), respectively; the majority of cows having two (83.3%, n = 18) and heifers three (64.7%, n = 16) waves (Figueiredo et al. 1997). Similar differences in oestrous cycle length between two and three wave animals have been reported in Holstein cattle (Sartori et al. 2004). Other studies in *B. taurus* have described the occurrence of two to four follicular waves during the oestrous cycle, with predominance of two waves, and very rarely four waves (Sirois & Fortune 1988, Townson et al. 2002). These authors also reported that 70% of Nelore females (n = 10) observed during two consecutive oestrous cycles repeated the same pattern of follicular waves of the previous cycle (i.e., two or three waves). After evaluating 117 interovulatory intervals (oestrous cycles) from 17 Brahman heifers, Rhodes et al. (1995) recorded that 26.5%, 66.7%, and 6.8% of cycles had either two, three, or four follicular waves per cycle, respectively; and the oestrous cycle length lasted, on average, 20.9 days. In five Brahman heifers examined over more than 12 oestrous cycles, it was reported that the same follicular wave pattern was repeated 60% to 87.5% of the times. Other studies in Nelore heifers (Sartorelli et al. 2005, Mollo et al. 2007), Gir cows (Gambini et al. 1998, Viana et al. 2000) and Brahman cows (Zeitoun et al. 1996) have reported a predominance of three wave cycles but also observed animals with two, four, and even a few with five waves per oestrous cycle.

The average ovarian antral follicle count (AFC) in *B. indicus* is twice the number observed for *B. taurus* (Fig. 28.2; Table 28.1). For example, at wave emergence the number of 2 to 5 mm follicles present in the ovaries was 42.7 (ranging from 25–100) in Nelore and 19.7 (ranging from 5–40) in Holstein cows (Bastos et al. 2010). Greater AFC has been associated with greater circulating anti-Müllerian hormone (AMH) in *B. indicus* compared with *B. taurus* (Baldrighi et al. 2014, Batista et al. 2014).

Another observed difference between *B. taurus* and *B. indicus* was the size of the largest follicle at deviation. In Holstein cattle the diameter of the future ovulatory follicle at the time of deviation was between 8.3 and 9.8 mm (Ginther et al. 1996, Sartori et al. 2004, Bastos et al. 2010). In Nelore heifers, deviation happened when the largest follicle reached 5.4 to 6.2 mm (Sartori et al. 2005, Gimenes et al. 2008) and in nonlactating Nelore cows between 7.0 and 7.4 mm of diameter (Bastos et al. 2010, Sartori et al. 2016). Nevertheless, the time after ovulation or after wave emergence for follicle deviation was similar for *B. taurus* and *B. indicus* (Sartori et al. 2010, Sartori et al. 2016) due to a slower growth rate of the follicles in Nelore (0.8–1.2 mm/day; Gimenes et al. 2008, Sartori and Barros 2011) compared with Holstein cattle (1.2–1.6 mm/day; Sartori et al. 2001).

Although follicle deviation occurs in *B. indicus,* when the dominant follicle reaches 5 to 7.5 mm in diameter compared with 8 to 10 mm in *B. taurus,* it is possible that additional growth is necessary for the dominant follicle to acquire ovulatory capacity in both breeds. Sartori et al. (2001) observed that Holstein cows with follicles 7 or 8.5 mm in diameter did not ovulate, even after administration of high doses of pLH (40 mg). However, 80% of the cows with follicles equal to or larger than 10 mm ovulated after pLH administration. Conversely, Gimenes et al. (2008) reported that administration of 25 mg pLH to *B. indicus* heifers induced ovulation in 33.3%, 80.0%, and 90.0% of animals, with follicles that were 7.0 to 8.4, 8.5 to 10 and larger than 10 mm in diameter, respectively.

The decrease in progesterone (P4) and increase in E2 near the time of ovulation are responsible for overt oestrous behaviour and

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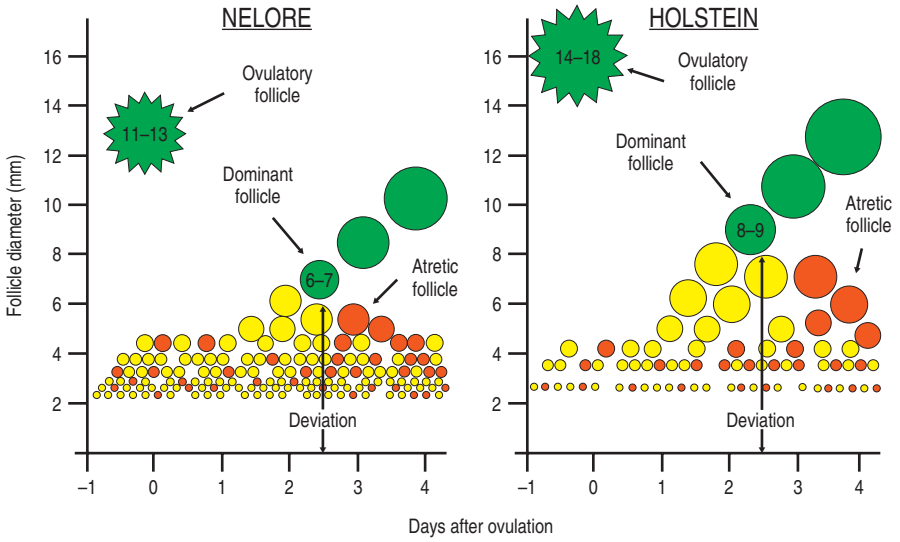

• **Fig. 28.2** Schematic representation of follicle development in Nelore (*B. indicus*) and Holstein (*B. taurus*) cows based on data from the literature and personal data. Nelore have a greater population of small (2–5 mm) follicles in the ovaries throughout the entire oestrous cycle than Holstein cattle. Moreover, although both breeds have follicle deviation between Day 2 and 3 after ovulation, the diameter of the future dominant follicle at deviation is greater in Holstein cattle. The diameter of the ovulatory follicle is also greater in Holstein than Nelore cows. (From Sartori et al. 2010.)

TABLE 28.1 Comparison of reproductive variables between *B. indicus* and *B. taurus*[a]

	B. indicus vs *B. taurus*
Oestrous cycle length	=
Day of luteolysis	≤
Number of waves per cycle	>
Ovarian antral follicle count	>
Circulating AMH	>
Size of dominant follicle at deviation	<
Day of deviation after ovulation	=
Growth rate of dominant/ovulatory follicle	<
Maximum size of dominant/ovulatory follicle	<
Oestradiol peak preceding ovulation	>
Duration of oestrus	≤
Luteal tissue volume	<
Circulating progesterone	>
Steroid hormones clearance rate	<
Circulating insulin	>
Circulating IGF1	>
Circulating FSH	≤
Circulating cholesterol	>

[a]From Segerson et al. (1984), Alvarez et al. (2000), Bó et al. (2003), Carvalho et al. (2008), Gimenes et al. (2008), Sartori et al. (2010), Sartori & Barros (2011), Sartori et al. (2013), Baldrighi et al. (2014), Batista et al. (2014), Sartori et al. (2016).

for the preovulatory GnRH/LH surge. Oestrus, the period of sexual receptivity, lasts between 30 minutes and 27 hours in *B. taurus* (Lopez et al. 2004) and between 1 and 20 hours in *B. indicus* (Bó et al. 2003). The onset of oestrus coincides with the preovulatory surge of LH followed by ovulation, on average, 26 to 28 hours later (Walker et al. 1996, Pinheiro et al. 1998, Mizuta 2003).

Studies in Nelore cattle have observed some specific characteristics regarding oestrus in this breed. The interval between the onset of oestrus and ovulation was 27.7 ± 2.4 and 26.1 ± 1.2 hours after $PGF_{2\alpha}$-induced and natural oestrus, respectively in nulliparous heifers, and in cows it was 26.8 ± 0.8 and 28.0 ± 0.9 hours after $PGF_{2\alpha}$-induced and natural oestrus, respectively (Pinheiro et al. 1998). Additionally, the oestrous behaviour, when detected (< 50% of the cows), was shorter (10.5 ± 1.0 and 13.6 ± 1.0 hours) after $PGF_{2\alpha}$-induced and natural oestrus, respectively compared with reports in European breeds, and there was a high incidence of oestrus at night.

Combined data from two other studies, in which oestrus was synchronised in 90 cyclic Nelore heifers and ovulation was confirmed by ultrasonography, were analysed. Although the majority of the heifers (76.7%) ovulated within 7 days after $PGF_{2\alpha}$ treatment, 50% of them were not observed in standing oestrus, even with continuous 24 hour-a-day visual monitoring along with the aid of heatmount detectors. Moreover, out of the 45 heifers that were not detected in oestrus, 25 (55.6%) ovulated, and only one (2.2%) that was seen in oestrus did not ovulate. Some of the possible reasons for the lack of oestrous behaviour in these studies, besides an inherent breed effect, were excessive animal handling for daily transrectal ultrasound evaluation and blood collection and/or a relatively steep fall in ambient temperature during some of the nights during the study.

One study that directly compared oestrous behaviour in Nelore cows with a European breed (Angus) was performed by Mizuta (2003). Using a radiotelemetric oestrous detection system (HeatWatch), the study verified that average oestrous duration in Nelore was shorter than in Angus cows (12.9 ± 2.9 vs 16.3 ±

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4.8 hours). However, the interval between the onset of oestrus and ovulation was similar: Nelore 27.1 ± 3.3 hours and Angus 26.1 ± 6.3 hours.

The effect of a high percentage of Nelore females displaying no signs of oestrus before ovulation, short oestrous duration, and a high percentage of cows that exhibit the start and end of standing oestrus during the night makes it difficult to detect signs; this, consequently, impairs the efficacy of AI in Nelore cows. This can be significantly reduced if FTAI, without the need for oestrous detection, is performed. Consequently, several hormonal treatments have been developed to allow FTAI in zebu cattle, including protocols for FTAI in embryo donors (Barros & Nogueira 2001, Fernandes et al. 2001, Baruselli et al. 2004, Bó et al. 2007).

The diameters of the largest ovulatory follicle and of the corpus luteum (CL) also differ between *B. taurus* and *B. indicus*. The ovulatory follicles of *B. taurus* have a larger diameter (see Fig. 28.2) and greater CL volume than *B. indicus*. However, circulating concentrations of steroid hormones in *B. taurus* are lower than in *B. indicus* (Sartori & Barros 2011, Sartori et al. 2016). In studies with Nelore cattle the diameter of the ovulatory follicle was 11 to 14 mm (Figueiredo et al. 1997, Sartorelli et al. 2005, Sartori et al. 2016), and the maximum diameter of the ovulatory follicle in Holstein cattle was 13 to 19 mm (Ginther et al. 1989, Sartori et al. 2002, Sartori et al. 2004). Similarly, the CL diameter of *B. indicus* ranged from 17 to 22 mm or approximately 2.5 to 5.0 cm^3 (Segerson et al. 1984, Rhodes et al. 1995, Figueiredo et al. 1997, Bó et al. 2003, Sartori et al. 2016), whereas the CL diameter of *B. taurus* ranged from 20 to 30 mm or approximately 4.0 to 14.0 cm^3 (Ginther et al. 1989, Bó et al. 2003, Sartori et al. 2004, Sartori et al. 2016).

Despite having larger follicles and CL, *B. taurus* have lower circulating steroid hormone concentrations. For example, nonlactating Holstein cows had larger ovulatory follicle diameter (14.2 vs 12.9 mm) and CL volume (5.2 vs 3.9 cm^3) than nonlactating Nelore cows (Sartori et al. 2016); however, Holstein cows had a lower preovulatory peak of circulating E2 (12.5 vs 16.2 pg/mL) and circulating P4 concentrations (1.9 vs 2.7 ng/mL) on Day 7 of the oestrous cycle, compared with Nelore cows.

This inverse relationship between the size of ovarian structures and circulating steroid hormones may be related to the reported differences in circulating insulin, IGF1, and cholesterol between breeds. Most of the studies have described greater circulating insulin and IGF1 in *B. indicus* than in *B. taurus* (Alvarez et al. 2000, Sartori & Guardieiro 2010, Sartori et al. 2013). Moreover, it has also been reported that, under similar nutritional management conditions, Nelore heifers have approximately 60% greater circulating cholesterol (precursor for the biosynthesis of steroid hormones) than Holstein heifers (196.8 vs 123.5 mg/dL; Gandra et al. 2011). Besides greater production of steroid hormones, there may be also reduced metabolic clearance rate for E2 and P4 in *B. indicus* compared to *B. taurus* (Sartori et al. 2016).

Nutritional and Metabolic Effects on Normal Reproductive Physiology and Reproductive Performance

The body condition score (BCS) in the first weeks postpartum exerts influences on metabolic hormones such as IGF1, insulin, glucose, NEFA, and thyroxine in *B. taurus* (Ciccioli et al. 2003). The same authors also reported that cows that maintain or lose BCS during lactation had a longer interval between calving, delayed first oestrous manifestation, and lower fertility (Ciccioli et al. 2003). In addition, *B. taurus* crossbreed cows (Charolais x Angus) maintained on pasture with BCS higher than 3 at calving had a lower postpartum anoestrous period compared with cows with BCS of 3 or lower, which presented the first ovulation postpartum later than 80 days (Houghton et al. 1990). These studies have shown that cows with moderate to good BCS at calving had a faster resumption of cyclicity (Whitman 1976, Graham 1982). In fact, in beef cattle, cows that lost less BCS before calving had fewer days open and higher pregnancy rates (Selk et al. 1988).

Few studies have directly evaluated the influence of nutrition on the reproductive variables of *B. taurus* versus *B. indicus*. In one study, using nonlactating Nelore (n = 12) and Holstein (n = 12) cows submitted to high or low feed intake, in a completely randomised design with a 2 x 2 factorial arrangement of treatments, cows were randomly assigned into two groups within each breed receiving 180% (1.8 M) or 70% (0.7 M) of a maintenance diet in a crossover design. Two weeks after the start feeding experimental diets, oestrus was synchronised and the cows were monitored twice a day by ovarian ultrasonography and blood samples collected once a day for hormone assays. Independent of diets, Holstein had fewer follicles at wave emergence than Nelore cows (12.7 ± 5.8 vs 31.8 ± 5.7 AFC). Results regarding all variables related to the size of ovarian structures were consistent with previous reports, in which Holstein cows had larger ovulatory follicle diameters (14.2 ± 0.4 vs 12.9 ± 0.4 mm) and CL volume (5.2 ± 0.3 vs 3.9 ± 0.3 cm^3) than Nelore cows. However, Holsteins had lower preovulatory peaks of circulating E2 (12.5 ± 1.4 vs 16.2 ± 1.3 pg/mL), plasma P4 concentrations on D7 (1.9 ± 0.3 vs 2.7 ± 0.3 ng/mL), and plasma insulin on D7 (2.3 ± 1.9 vs 7.6 ± 1.8 µIU/mL) than Nelore cows. Independent of breed, overfed cows had larger ovulatory follicle diameters (14.6 ± 0.3 vs 12.4 ± 0.3 mm) and larger CL volumes (5.2 ± 0.3 vs 3.9 ± 0.3 cm^3). However, they had lower plasma E2 preovulatory peaks (12.7 ± 1.2 vs 16.0 ± 1.3 pg/mL) and plasma insulin values on D7 (7.2 ± 1.6 vs 2.6 ± 1.9 µIU/mL) than those in low feed intake, although plasma P4 concentrations on D7 did not differ (2.2 ± 0.2 vs 2.4 ± 0.2 ng/mL, high vs low, respectively) between diets. Interestingly, regardless of genetic group, overfed cows had follicle deviation occurring later in the cycle (2.9 ± 0.2 vs 2.5 ± 0.2 days after ovulation), and the diameter when the largest follicle reached deviation was also greater (8.1 ± 0.4 vs 7.4 ± 0.4 mm) than those on low feed intake. Finally, independent of all the differences observed between breeds, the effect of nutrition on the analysed variables was similar for Nelore and Holstein cows.

It is known that the liver metabolism of steroid hormones is correlated with the dry matter intake (Sangsritavong et al. 2002, Vasconcelos et al. 2003). However, studies in which cows were fed the same maintenance diet and dry matter intake per kilogram of body weight was 1.37% for Nelore and 1.54% for Holstein cows (according to NRC 2001 recommendations), there was no body weight gain or loss in any of the studies for both breeds. Therefore, based on these results, a possible higher steroid metabolism in Holstein cows may be inherent to the breed and not only a consequence of higher feed intake. Moreover, despite contrasting data from previous studies (Randel 1976, Segerson et al. 1984), tropical cattle (mainly represented by the Nelore breed) may have lower liver steroid metabolism and increased steroid production by the ovaries than temperate cattle (represented here by the Holstein breed); this may be associated with higher circulating insulin and IGF1 in *B. indicus*.

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Influence of Dry Matter or Energy Intake Associated With High Plasma Insulin Concentrations on the Yield and Quality of Embryos

Most researchers who have studied the effect of feed intake on embryo production of *B. taurus* cows or heifers have reported negative results on the reproductive function of overfed cattle compared with those fed restricted diets. The causes of impaired embryo production related to feed intake are still not well understood. However, changes in liver blood flow, local and circulating metabolites (glucose and IGF1), hormone concentrations (insulin and steroids), and different sources of volatile fatty acids may be involved in these processes. Moreover, we hypothesised that the effects of dietary intake on embryo quality may differ between *B. taurus* and *B. indicus,* which consistently show different concentrations of circulating insulin and IGF1 concentrations. As shown later in this chapter, it is tempting to speculate that Zebu cattle may be more resistant than European breeds to the effects of changes in feed intake on embryo production and quality.

Bastos et al. (2007) did not detect any effect on the superovulatory response, embryo production, or embryo quality in Nelore heifers (n = 36) with higher or lower BCS fed maintenance (M) or flushing (1.8 M) diets for 14 days before the superovulatory treatment. However, there were clear differences in nutritional effects on cows subjected to OPU and *in vitro* production (IVP) of embryos compared with superovulation and *in vivo* development of embryos (Sartori et al. 2017). Thus all cows went through all treatment groups (n = 32 per group) and both types of embryo production systems.

Embryos (n = 274 vitrified embryos) were subsequently transferred to evaluate the effects of *in vivo* treatments on subsequent pregnancy per fixed-time embryo transfer (P/ET) in Nelore recipients. As expected, preprandial circulating insulin measured in blood collected on Day 32 (at the onset of superstimulation treatments) was greater in the E group compared with the 0.7M and M groups (8.7 ± 0.9, 4.6 ± 0.9, and 5.3 ± 0.85 μIU/mL respectively), but did not differ from that in the 1.5M group (6.6 ± 0.9 μIU/mL). Superovulation (as determined by the number of CL) was less in donors fed the E diet compared with cows receiving the standard (0.7M, M and 1.5M) diets (9.7 ± 1.2, 13.0 ± 1.3, 14.2 ± 1.2, and 13.9 ± 1.2, respectively), with a negative correlation between circulating insulin and CL number ($r = -0.32$). Nevertheless, there was no difference between groups for number of ova or embryos (~6), viable embryos (~3), or freezable embryos (~2.7). Regardless of treatment, circulating insulin was negatively correlated with the number of viable embryos (r = −0.22). There was a lower P/ET at 60 days in the 97 cows receiving embryos from donors fed the E diet (29.4%) compared with the 177 cows receiving embryos from donors fed the standard (0.7M, M or 1.5M) diet (43.3%). Moreover, probability of P/ET at 60 days, evaluated by logistic regression, decreased as circulating insulin concentrations of donor cows increased from 0.64 to 25.0 μIU/mL. Therefore high circulating insulin in the donors was associated with a reduced superovulatory response, fewer viable embryo, and poorer P/ET at 60 days.

In the same study for IVP, cows with low energy intake (0.7M) differed from cows fed the M or E diets, but not those fed the 1.5M diet (which exhibited intermediate values), with more oocytes recovered (23.0 ± 2.3, 20.2 ± 2.0, 21.5 ± 2.2, and 20.1 ± 2.0 for the 0.7M, M, 1.5M, and E groups respectively), and a greater number of viable oocytes per OPU (17.0 ± 1.9, 14.4 ± 1.6, 15.7 ± 1.7, and 14.1 ± 1.6 for the 0.7M, M, 1.5M, and E groups respectively). The number of cleaved oocytes per OPU was also greater in cows fed the 0.7M diet compared with the M diet (13.4 ± 1.7, 10.7 ± 1.4, 12.6 ± 1.6 and 11.7 ± 1.5 for the 0.7M, M, 1.5M, and E groups, respectively). However, the study did not detect an effect of diet on the number (6.9 ± 0.9, 5.4 ± 0.8, 5.9 ± 0.8, and 6.6 ± 0.9 for the 0.7M, M, 1.5M, and E groups respectively) or percentage (30.6, 31.9, 31.1, and 34.0% for the 0.7M, M, 1.5M, and E groups respectively) of blastocysts produced *in vitro.* Thus negative effects of high energy intake appear to be exerted at the later stages of follicle development or during early embryo development, and not during the early stages of follicle development, because high energy negatively affected embryo production and quality in cows that were superovulated and underwent *in vivo* embryo production but not in the case of oocytes collected from small follicles.

However, another study (Sales et al. 2015) has shown that nonlactating Gir cows, in which 1.7M diet was offered for more than 60 days, had a decrease in blastocyst rate over time (46.9 vs 25.7%). Nevertheless, in the same study, blastocyst rate was the same in nonlactating Holstein cows before or after being overfed for more than 60 days (6.5 vs 9.8%).

With the purpose of investigating the effect of fat supplementation on embryo production, Guardieiro et al. (2014) supplied concentrate with or without rumen-protected fat (Megalac-E, Elanco Animal Health, São Paulo, Brazil), rich in linoleic acid, to 40 Nelore heifers, starting 50 days before superovulation in a crossover experimental design. Supplemental diets were isocaloric and isonitrogenous. The superstimulatory response, the number of total embryos/ova, viable embryos, degenerate embryos, or unfertilised oocytes recovered were similar between groups. However, there was negative effect of unsaturated fatty acids on the superovulatory response (15.7 ± 1.2 vs 18.0 ± 1.3 CL), hatching rate at 48 hours (17.3 ± 3.3%; n = 137 vs 33.1 ± 4.0%; n = 148), and at 72 hours (30.9 ± 4.0%; n = 137 vs 44.3 ± 4.2%; n = 148) of *in vitro* culture after thawing. This negative effect associated with a rumen bypass fat diet may have been influenced by lower plasma concentrations of IGF1 observed in this experimental group compared with control (374.3 ± 27.2 vs 483.8 ± 26.5 ng/mL, respectively).

Assisted Reproductive Technologies (ARTs) Applied to *B. indicus*

Most of beef cattle herds in the tropics, especially in Brazil, are composed of *B. indicus* that have a longer postpartum anoestrus and low BCS when kept at pasture (Bó et al. 2003). This results in economic losses because of the increased calving to conception interval and reduced pregnancy rates (PR; Bó et al. 2007). In a pasture-based cow–calf production system, the use of reproductive management programmes, such as synchronisation of ovulation for FTAI (synchronisation protocols based on P4 and E2), is essential to achieve high PR in the breeding season, and it has been increasingly incorporated in cow–calf operations (Pessoa et al. 2016).

Data generated by the GERAR group (Specialised Group in Applied Reproduction to the Herd) in Brazil show the improvements in pregnancy rates from AI using P/AI from 2007 to 2015 in millions of heifers and cows submitted for FTAI. The main FTAI protocol used during the period of the study was the following: (1) D-11: Progesterone insert + 2 mg EB; (2) D-4: 12.5 mg dinoprost trimethamine; (3) D-2: P4 insert removal + 0.6 mg

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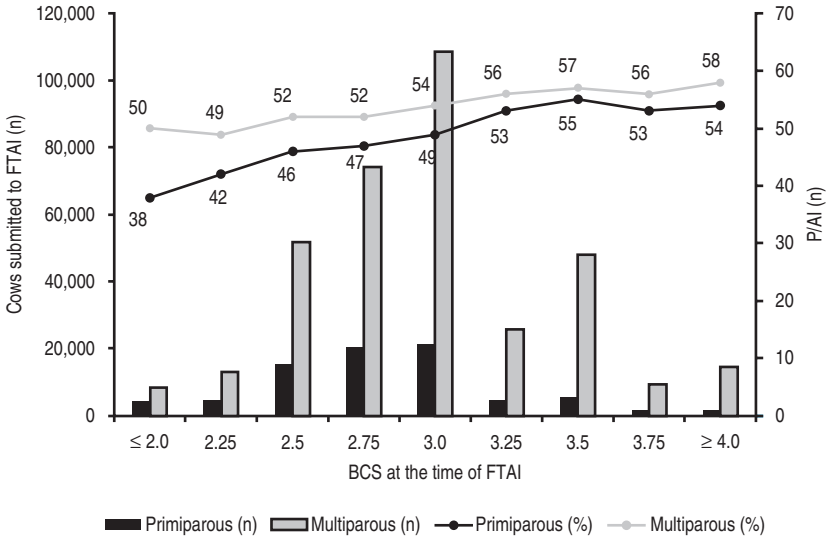
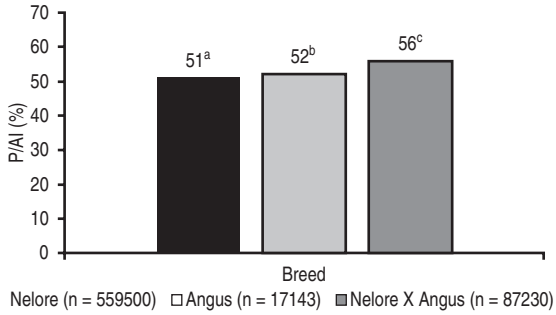


• **Fig. 28.3** Number and P/AI of primiparous and multiparous beef cows submitted to FTAI according to BCS.

ECP + 300 IU equine chorionic gonadotropin (eCG) or calf removal for 48 hours; (4) D-0; FTAI (Meneghetti et al. 2009, Peres et al. 2009, Sa Filho et al. 2009). The fertility in all types of beef cattle has been relatively constant (~50%) during recent years. Nevertheless, there is likely to be room for improvement in many of the herds because some herds (~24%) had average P/AI greater than or equal to 60%. These herds are likely to have more intensive reproductive programmes: better nutrition with fewer cows with low BCS that could impact on fertility, especially in primiparous cows (Fig. 28.3); the cattle may also have better fertility traits, such as Nelore x Angus crossbreeds that also have an effect on P/AI results (Fig. 28.4).

Sex-sorted sperm has been widely and increasingly used in Brazil, especially for AI or IVP. Unfortunately, epidemiological data on the use of sex-sorted sperm in Brazil are not available.



• **Fig. 28.4** Breed effect on P/AI of cows submitted to FTAI. ($^{a,b,c}P < 0.01$.)

Breeding Season

One of the strategies commonly used for reproductive management of *B. indicus* is the establishment and incorporation of a breeding season (BS) in the management systems. A specific BS facilitates the concentration of calving and calf rearing, allowing breeding to occur when favourable nutritional and environmental condition for tropical environments are present, due to better forage availability. Usually, the BS occurs throughout spring and summer, lasting on average 3 months, and as a consequence, calving is concentrated during seasonal dry periods.

One of the best methods of increasing the number of females that are inseminated during the BS is the use of FTAI protocols. There are many strategies used in the reproductive management of *B. indicus* herds during the BS, with the objectives of improving pregnancy rates, accelerating genetic gain, and having as many females as possible pregnant at the end of the BS. Among the strategies that can be highlighted are: FTAI, improved oestrous detection, and use of natural service. Ideally, it is important to maintain a 365-day calving interval, in which cows conceive on average at least by 70 to 80 days postpartum.

Sá Filho et al. (2013) compared different reproductive management strategies during the breeding season and showed that cows submitted for their first postpartum AI after oestrous detection, followed by natural service, or just natural service during the entire BS had a decreased chance of pregnancy compared with cows in the two groups that had been bred only by FTAI at the onset of the BS. Moreover, cows bred by FTAI (~93%) had higher pregnancy rates at the end of the BS compared with cows not bred by FTAI (~84%). They also concluded that cows submitted for FTAI became pregnant sooner during the BS than cows submitted for AI after oestrous detection or natural service (Fig. 28.5).

Fixed-Time Artificial Insemination Protocols

The fixed-time artificial insemination (FTAI) protocol allows for a large number of cows to be inseminated on the same day, with high reproductive efficiency and at relatively low cost. Nevertheless, the success of the FTAI programme depends on several factors, such as synchronisation of follicular waves to optimise the period of follicular dominance, in order to reduce the chance of follicles ovulating too late (Cerri et al. 2009, Peres et al. 2009) or too early (Vasconcelos et al. 2001). This can be achieved by: synchronisation

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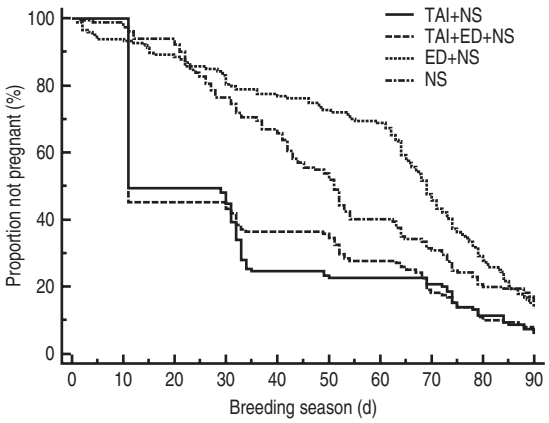

• **Fig. 28.5** Survival curves for proportion of nonpregnant cows by days of BS for various breeding strategies during a 90-day breeding season. TAI + NS (n = 150): cows received TAI on Day 11 of the BS, followed by NS until the end of the BS; TAI + ED + NS (n = 148): cows received TAI at Day 11, then were observed for oestrus twice daily, with AI 12 hours after ED until Day 45 of the BS, followed by NS until the end of BS; ED + NS (n = 147): cows were bred by AI 12 hours after ED during the first 45 days of the BS, followed by NS until the end of BS; NS (n = 149): cows were bred by NS throughout the BS. BS, breeding season; ED, oestrous detection; NS, natural service; TAI, timed AI. (From Sá Filho et al. 2013.)

of CL function and circulating P4; complete luteolysis at the end of the protocol; and synchronisation of final ovulation with optimally scheduled FTAI.

There are two main methods for the synchronisation of follicular wave emergence in dairy and beef cattle: (1) GnRH-based protocols, in which in the presence of a dominant follicle, GnRH induces ovulation and a new follicular wave begins; (2) E2/P4-based protocols, which after induced regression of all follicles, there follows a new wave commencing within the following days, depending on which E2 ester is used (Bó et al. 2003).

There are several factors that influence the proportion of cows ovulating after a GnRH/LH surge at the initiation of a FTAI protocol, such as the day of the oestrous cycle (Vasconcelos et al. 1999) or follicle diameter (Sartori et al. 2001, Gimenes et al. 2008). Although GnRH-based protocols have been successfully used in *B. taurus* cows (Pursley et al. 1995, Pursley et al. 1997, Bridges et al. 2008) but not necessarily in *B. taurus* heifers (Pursley et al. 1995, Pursley et al. 1997), there are conflicting data regarding their effectiveness in *B. indicus* cows (Fernandes et al. 2001, Bó et al. 2003, Baruselli et al. 2004, Ferraz et al. 2016).

Because it has been shown that *B. taurus* did not have consistent ovulations after GnRH treatment even in the presence of a dominant follicle larger than 10 mm in diameter (Martinez et al. 1999, Perry and Perry 2009), factors other than follicle size, such as circulating concentration of P4, may affect the ovulatory response to GnRH. For example, one study (Biehl et al. 2013) compared the response to the first GnRH (100 μg gonadorelin) treatment of the 5d CO-Synch + CIDR (5-d CS) programme in Nelore heifers. The treatments were: HiP4 (n = 62; GnRH in heifers with a CL present at P4 device insertion); LoP4 (n = 35; GnRH in heifers with no CL present at P4 device insertion); PGF-LoP4 (n = 65; GnRH in heifers that had a CL, but were treated with 25 mg of dinoprost (PGF) 2 d before GnRH treatment and P4 device insertion). Ovulation to GnRH was greater for the LoP4 (85.7%) and PGF-LoP4 (95.4%) compared with the HiP4 (25.8%). Moreover, it is possible that different analogues of GnRH may produce different outcomes because it has been reported, for example, that gonadorelin induced a lower LH surge compared with other analogues of GnRH, such as lecirelin, fertirelin, or buserelin (Chenault et al. 1990, Picard-Hagen et al. 2015).

Thus GnRH-based protocols are likely to result in poorer synchronisation of the first follicular wave when they are initiated in the presence of a CL, primarily due to lack of ovulation of a dominant follicle at the initiation of the protocol. This is clearly related to the greatly reduced magnitude of the LH surge, induced by GnRH in the presence of elevated P4. In addition, due to the natural growth and regression of follicular waves during the luteal phase, there are many times when an LH-responsive dominant follicle is not present, and therefore ovulation would not occur even with an optimised LH surge. Furthermore, the presence of elevated P4 may reduce LH receptor expression in the dominant follicle, and this could reduce responsiveness to an LH surge in dominant follicles (Dias et al. 2014).

Many GnRH-based protocols in *B. taurus* now use presynchronisation strategies to assure that cows are at a stage of the oestrous cycle that optimises ovulation to GnRH treatment. For example, double Ovsynch uses an Ovsynch protocol to presynchronise the cows, so that almost all cows (~94%; Herlihy et al. 2012) are cycling and at Day 6 or 7 of the oestrous cycle at the start of the breeding Ovsynch protocol. This produces a high ovulation rate to GnRH and high fertility during the protocol. These presynchronisation strategies have not been adequately tested in *B. indicus* but are likely to be too complicated for most *B. indicus* management systems. Unfortunately, studies have not yet been performed that directly tested the ovarian responses and fertility of *B. taurus* versus *B. indicus* in response to the same GnRH-based protocols.

Early studies that established the physiological basis for these protocols utilised *B. taurus* beef heifers and found that treatment with different esters of E2 simultaneously with P4 treatment led to suppression of the gonadotropins, regression of growing follicles, and emergence of a new follicular wave about 4 days after E2 treatment (Bó et al. 1993, Bó et al. 1994, Bó et al. 1995). Although E2/P4-based FTAI protocols are widely used in *B. taurus* and *B. indicus,* the metabolism of E2 is different between *B. taurus* and *B. indicus*. It was concluded that the timing of follicle wave emergence after treatment with EB + P4 was EB dose-dependent for both breeds.

The results presented previously are somewhat contradictory because, despite a very different clearance rate of E2, the behaviour of wave emergence after EB + P4 treatment was not different between breeds. Therefore based on differences in circulating E2 after EB treatment between breeds, one might expect that lower EB doses should be used for Nelore and higher EB doses should be used for Holstein cattle at the initiation of an E2/P4-based FTAI protocol in order to optimise synchronisation of follicle wave emergence. In contrast, based on the similar responses of both breeds to EB treatments in terms of follicle wave emergence, a similar E2 dose might be optimal for Nelore and Holstein cows.

Pessoa et al. (2015) compared 1 mg with 2 mg EB at the onset of a resynchronisation protocol starting 22 days (Day 22) after the first FTAI and evaluated P/AI, pregnancy loss and induction of new follicular wave emergence in 1426 suckled beef cows (768 *B.taurus* and 728 *B.indicus*). They concluded that 2 mg EB produced a more uniform emergence of the follicular wave and greater P/AI after resynchronisation without compromising the pregnancy established at the first FTAI.

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Regarding induced luteolysis by exogenous treatments, the responsiveness of the CL to $PGF_{2\alpha}$ seems to be very similar between *B. indicus* and *B. taurus.* To test the effect of the dose of a $PGF_{2\alpha}$ analogue and day of the oestrous cycle at the time of treatment, Ferraz et al. (2016) performed an experiment with nonlactating Nelore cows in a factorial design 3 x 2, in which three different doses of dinoprost tromethamine (12.5, 25, and 50 mg) were used on Days 5 or 7 of the oestrous cycle. In this study, luteolysis was dose-dependent. Therefore increasing the dose of $PGF_{2\alpha}$ increased the proportion of cows detected in oestrus and augmented the percentage of cows with circulating P4 concentration less than 1 ng/mL at 72 hours after $PGF_{2\alpha}$ treatment. In addition to the dose of $PGF_{2\alpha}$, it is well known that the day of the oestrous cycle influences the proportion of cows that undergo luteolysis after $PGF_{2\alpha}$ (dinoprost tromethamine) treatment. Ferraz et al. (2016) observed that Nelore cows, with a CL on Day 5 of the oestrous cycle, were more refractory to $PGF_{2\alpha}$ than those on Day 7 of the cycle. Moreover, in lactating Holstein cows a second treatment of dinoprost tromethamine (+ $PGF_{2\alpha}$; 24 hours apart) increased the percentage of cows that had complete luteal regression (95.6%), compared with control cows (84.6%). Although one study found no detectable effect of the additional $PGF_{2\alpha}$ on P/AI (control = 41.5% vs + $PGF_{2\alpha}$ = 44.7%; P = 0.34; Brusveen et al. 2009) and recent studies have found significant improvements in P/AI when a second $PGF_{2\alpha}$ treatment is given, particularly in multiparous cows (Carvalho et al. 2015, Wiltbank et al. 2015). Therefore complete luteolysis is essential to optimise ovulation and fertility in a FTAI protocol. In fact, more lactating Holstein cows with P4 < 0.1 ng/mL, compared with P4 of 0.1 or higher and less than 0.22 ng/mL at the time of AI, ovulated to a E2/P4-based FTAI protocol (81.2 vs 58.0%) and had increased P/AI (47.4 vs 21.4%; Monteiro et al. 2015). Similar results were observed by Pereira et al. (2013) when lactating dairy cows were also synchronised with an E2/P4-based protocol and were either inseminated or served as embryo recipients (ET). The P4 concentration at the time of AI or 7 days before ET altered fertility in FTAI cows with higher P/AI in cows with P4 concentration less than 0.1 ng/mL compared with cows with P4 concentration of 0.1 ng/mL or more, and in ET cows with higher P/ET in cows with P4 concentration less than 0.22 ng/mL compared with cows with P4 concentration of 0.22 ng/mL or higher.

Embryo Technologies

The physiological differences between *B. indicus* and *B. taurus* described previously need also to be considered when managing females for *in vivo*–derived (IVD) embryos by multiple ovulation and embryo transfer (MOET) or *in vitro* embryo production (IVP).

One of the protocols most frequently used in *B. indicus* cows and heifers for MOET was developed by Barros and Nogueira (2005) and was called P-36. The protocol included the use of an intravaginal P4 device for 36 hours after $PGF_{2\alpha}$ administration and the induction of ovulation with exogenous LH, administered 12 hours after the P4 device removal (48 hours after $PGF_{2\alpha}$ administration). The FTAI was performed 12 and 24 hours later because ovulation occurs between 24 and 36 hours after LH administration. The effectiveness of the P-36 protocol has been confirmed (Baruselli et al. 2006, Nogueira et al. 2007). A variation of the P-36 protocol, in which the P4 device is removed 24 hours after $PGF_{2\alpha}$ (protocol P-24) and LH is administered 24 hours later (48 hours after $PGF_{2\alpha}$), has been utilised in Nelore cattle, apparently with comparable results to those obtained with P-36 protocol (Baruselli et al. 2006).

The use of the P-36 protocol in *B. taurus* breeds, however, has resulted in decreased numbers of viable embryos in comparison with conventional protocols using oestrous detection. In Holstein (Baruselli et al. 2006) and Angus (Bó et al. 2006) donors, viable embryo production was increased with the P-36 protocol, when the ovulation induction treatment (LH or GnRH) was administered at 60 hours (P-36/LH60) rather than at 48 hours (P-36/LH48) after $PGF_{2\alpha}$ administration. On the other hand, even though delaying ovulation for 12 hours in the P-36 protocol in *B. taurus* breeds (P-36/LH60 protocol) had positive effects on embryo production, the opposite occurred when used in *B. indicus* breeds. The P-36/LH60 protocol caused a decrease in embryo production compared with P-36/LH48 protocol (Baruselli et al. 2006). Therefore it can be inferred that ovulation in superstimulation protocols must be induced earlier in *B. indicus* donors, whereas in *B. taurus* donors, it seems necessary to delay treatment with an ovulation inducer, thereby allowing an increase in follicle size and acquisition of LH receptors. *Bos indicus* breeds have a reduced capacity for LH secretion and a greater sensitivity to exogenous gonadotropins than *B. taurus* (Randel 1984).

Because of the greater AFC and better oocyte quality in *B. indicus* in comparison with *B. taurus,* IVP is much more successful in *B. indicus.* For example, Gir (*B. indicus*), Holstein, and crossbreeds (1/4 Holstein x 3/4 Gir or 1/2 Holstein-Gir) were compared for total and viable oocyte yield and IVP of embryos (Pontes et al. 2010). The number of total and viable oocytes and embryos produced was greater in Gir than in Holstein cattle (17.1 vs 11.4; 12.1 vs 8.0; 3.2 vs 2.2, respectively). Moreover, embryo production (5.5 blastocysts) was even greater in Holstein-Gir crossbreeds compared with the other breeds. Another study was conducted to compare IVP between Nelore and Holstein heifers (Gimenes et al. 2015). More oocytes were recovered (37.1 vs 15.4), more embryos were produced (7.3 vs 1.1), and a greater blastocyst rate was obtained (28.3% vs 14.1%) from Nelore than Holstein heifers. In another study (Sales et al. 2015), Gir cows had a greater number of oocytes recovered by ovum pickup (OPU; 23.4 vs 14.9), better quality of oocytes as demonstrated by greater cleavage rates (73.6% vs 40.8%), greater number of blastocysts (3.8 vs 0.7), and better blastocyst rates (36.7% vs 12.1%) than Holstein donors.

Overall, the main findings of the studies described previously indicate that *B. taurus* yielded fewer oocytes as well as produced fewer blastocysts per OPU than *B. indicus* donors. This pattern seems to occur even when other *B. taurus* breeds are studied. Sudano et al. (2014) conducted an experiment in which nonlactating Simmental (*B. taurus*) and Nelore cows were compared for *in vivo* embryo production and IVP. The total number of recovered ova/embryos per cow (5.5 vs 3.7) and transferable IVD embryos per cow (3.8 vs 2.3) were not different between Nelore and Simmental, respectively; however when IVP was performed, Nelore produced more oocytes per OPU (14.9 vs 8.1) and had greater blastocyst rates per OPU (41.5% vs 23.4%) than Simmental cows.

Nelore cows (with or without a calf) were synchronised with the same protocol within a 3-month period. For FTAI, 346 cows were bred on Day 0 using frozen/thawed semen of five bulls. For ET, cattle received IVD (n = 274) or IVP (n = 573) vitrified embryos (produced with semen from seven bulls, of which three were the same bulls used for FTAI) on Days 6, 7, or 8 of the protocol after confirming the presence of a CL. The same groups of cows were used for all treatments. When this variable was used for analysis, more healthy calves were born per cow submitted to a synchronisation protocol for the FTAI group and less for the IVP group (Table 28.2).

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TABLE 28.2 Pregnancy per AI or P/ET, pregnancy loss, abortion, and peripartum loss in Nelore cows that received fixed-time AI (FTAI) or vitrified *in vitro* produced (IVP) or *in vivo* derived (IVD) embryos

	FTAI	IVD	IVP
30 d pregnancy, % (n/n)	50.3[a] (174/346)	39.4[b] (108/274)	34.0[b] (195/573)
60 d pregnancy, % (n/n)	47.7[a] (165/346)	35.4[b] (97/274)	28.6[c] (164/573)
Embryo/foetal loss (30–60 d), % (n/n)	5.2[b] (9/174)	10.2[ab] (11/108)	15.9[a] (31/195)
Later pregnancy loss (60 d to calving), % (n/n)	15.2[a] (25/165)	6.3[b] (6/96)	16.5[a] (27/164)
Peripartum loss, % (n/n)	2.1[b] (3/140)	4.4[ab] (4/90)	9.5[a] (13/137)
Total loss, % (n/n)	21.3[b] (37/174)	19.4[ab] (21/108)	36.4[a] (71/195)
Healthy calf born per synchronization protocol, % (n/n)	39.6[a] (137/346)	25.4[b] (87/342)	17.3[c] (124/716)

[a,b,c]Means with different superscripts within the same line differ ($P < 0.05$).

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Infertility and Abortion in Sheep and Goats

PEERS DAVIES

Sheep

Three factors influence the numbers of lambs sold: fertility, i.e., whether the ewes are pregnant and lamb; fecundity, i.e., the number of lambs born per pregnancy; and survival rate of lambs to weaning.

The level of fertility in sheep is usually expressed as the reproductive performance of the flock. This can be defined as the number of lambs born per 100 ewes put to the ram (i.e., true lambing percentage). The breeding season commences with the introduction of rams, and all physical and financial performance should be calculated from this point, taking into consideration ewes that die, those that are culled, and those that abort or are barren. Empty ewes are a cost to the flock but are often not included in costings, making between-flock comparisons difficult and, in some cases, giving an overoptimistic impression of reproductive performance when the denominator for lambing percentage only includes those that have conceived, as measured at scanning, or those which gave birth to lambs. Attention should be paid to both the empty ewe % at pregnancy diagnosis (midpregnancy) and the aborted % that itself includes both the observed abortions and also unobserved abortions (those ewes that were confirmed pregnant and subsequently did not lamb, sometime termed 'wet-to-dry' ewes). Ewe conception rate per cycle is normally accepted to range from 80% to 90% in well managed flocks during the prime breeding season for the breed. This is commonly calculated from lambing records with the assistance of raddle mark information in some cases. Based on this conception rate after a mating period of two cycles, it would be expected that the physiological empty rate should not exceed 4% of the flock or 0.8% after 3 cycles.

The most recent published figures for ewe productivity in lowland (non-SDA) and upland/hill (SDA) flocks in England published in 2016 revealed scanning percentages of 170% and 164% respectively with empty rates of 6% and 4.6% respectively. The abortion rates (observed and unobserved) were 10% and 6% respectively (AHDB Stocktake Report 2016). However, the observed abortion rate is typically far lower than this given the extensive nature of most sheep production systems in the UK and worldwide. Empty or aborted ewes are frequently culled and, as a consequence, there is some genetic selection against poor fertility. Fecundity is influenced by genetic selection, age of the ewe, nutritional status, and environment. Lamb survival rate will be influenced mainly by management factors, the environment, and also genetic selection for such traits as good mothering behaviour.

Ewes are generally allowed to run with the ram during the breeding season and not segregated; thus oestrus detection problems are not frequently encountered. Furthermore, most breeds of sheep have a long period of acyclicity after parturition, allowing the reproductive system time to recover from the effects of pregnancy. Published information on normal conception rates in British lowland ewes is vague. However, the fertility of ewes, as measured by pregnancy (conception) rates to first service by Smith (1991) was 91.6%. In the same study, 99.4% had conceived by the third mating. Conception rates are influenced by a number of physiological and managemental parameters as well as pathological processes that may prevent conception but are more commonly associated with abortion. Structural or anatomical factors are of limited importance.

True anoestrus is uncommon in sheep; Smith (1991) identified the condition in 0.3% of 5488 lowland ewes. In fact, when the rams are turned out with the flock, it is usual for most of the ewes to be mated within a month. The first oestrus of the breeding season in some ewes is anovulatory and, according to Dutt (1954), more frequently ewes fail to become fertilised at these early matings compared with later ones. Ovarian follicular cysts, commonly encountered in cattle, are of limited importance in sheep. Smith (1996) identified follicular cysts in 2.9% and 10.02% of abnormal parous and nulliparous genital tracts respectively. Luteal cysts were rare.

Embryonic death, or resorption, is a conspicuous feature of sheep infertility and is more often associated with multiple than with single conception. It is suggested that a greater degree of embryonic death follows early matings. Using laparoscopy and comparing the number of corpora lutea with the number of fetuses the incidence of the condition has currently been estimated at 20% to 33% (Wallace & Ashworth 1990, Bruere & West 1993).

Early embryonic death has also been associated with infectious diseases such as toxoplasmosis, Border disease, and Schmallenberg disease (see later in this chapter). In a survey by Johnston (1988), 35.2% of barren ewes had elevated antibody titres to *Toxoplasma* compared with 19.2% of fertile ewes.

Noninfectious Causes

Nutrition

Ewe nutrition plays an important role in both fertility and fecundity. The effects of nutrition on fertility and fecundity

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are not restricted to the period around mating but are influenced by the nutritional experience of the individual during its own development. For example, recent research in ewes has demonstrated that during its early development the fetal ovary is remarkably sensitive to maternal nutrition with subsequent lifetime effects on ovulation rate (Robinson et al, 2006). The timing of puberty in both sexes and adult ovulation rates in ewes are influenced by postnatal nutrition. Nutrition during the period when ovarian follicles emerge from the primordial pool (approximately 6 months before they ovulate in ewes) can influence ovulation rate in ewes. The role of nutrition can be considered in the form of energy, micronutrients/trace elements, and finally bioactive chemical compounds that act directly upon the reproductive tract derived principally from varieties of legumes, especially red clover grown as a high protein, draught tolerant, nitrogen-fixing forage crop for grazing or conservation as hay or silage.

Energy

It is important that ewes are in good bodily condition at mating. Increasing the energy intake several weeks before tupping so that the ewes are gaining weight (flushing) has been common practice for decades as a means to increase the fecundity in those ewes with the genetic potential. However, it has been demonstrated that maintaining ewes at body condition score (BCS) 3.5 to 4 (1–5 scale) before mating has the same effect as 'flushing' lower BCS ewes by increasing the energy intake in and weeks preceding mating. Studies conducted in Australian sheep flocks have demonstrated a normal distribution of fetal numbers with BCS an optimum conception rate at a BCS of 3.5 determined by ultrasound pregnancy diagnosis.

Trace Elements

It has been demonstrated that the cobalt/vitamin B12 status of donor ewes in superovulation protocols affected ovulation rate. In a study by (Mitchell et al. 2007), ewes with mean circulating B12 concentrations of 1483 and 182 pmol/l had mean ovulation rates of 14.4 ± 1.28 and 9.9 ± 1.55, respectively.

Selenium has been demonstrated to have a significant effect on ram fertility (Kendall et al. 2000); however, the significance in ewes is less clear. The importance of copper and zinc to fertility and fecundity is also unclear; however, in the developing fetus the importance of sufficient bioavailable copper in the maternal diet is well known in order to prevent inadequate axonal myelination leading to enzootic ataxia in lambs 'swayback'.

Iodine supplementation of ewes and rams grazing severely deficient pastures has been associated with a significant improvement in fertility (Ferri et al. 2003, Clark et al. 1998). Iodine sufficiency of the maternal diet has also been associated with lamb survival (Clark et al. 1998).

Bioactive Agents – Red Clover

Since the 1950s, studies conducted in Australia have demonstrated temporary and, in some cases, permanent infertility induced in ewes by the prolonged grazing of red clover pasture. This led to a number of chemical and biological studies, mostly conducted on red clover grown in Australia, indicating that red clover contained oestrogen-like compounds (Marley 2011). Later, researchers identified that red clover with high levels of the phytooestrogens formononetin (7-hydroxy-4'-methoxyisoflavone) and biochanin A (5,7-dihydroxy-4'-methoxyisoflavone) indirectly caused the infertility.

Sheep grazing pastures with red clover at less than 25 % of total dry matter could not be guaranteed not to cause fertility problems as it has been shown that sheep will selectively graze clovers over grasses in mixed swards.

The phytooestrogen content of red clover is primarily under genetic control, but environmental factors can also have an effect. Different cultivars have been shown to contain different concentrations of phytooestrogens (Marley 2011), and plant breeders have developed many new varieties of red clover, recognised for their lower levels of the oestrogenic compounds (e.g., the variety AberRuby). In Australia and New Zealand, pasture contains higher levels of phytooestrogen in spring, with oestrogen content declining after flowering. Nutrient deficiencies that impair plant growth have also been shown to increase the concentrations of formononetin, and hay and silage have been shown to retain oestrogenicity in some cases (Marley 2011). In particular, one study highlighted that the oestrogen content of red clover silage was higher than that of the raw material and that the type of silage inoculant used during ensiling could affect phytooestrogen levels (Sarelli et al. 2003).

Management Factors

The management of ewes before, during, and after mating can all play a key role in the reported fertility and fecundity of the flock. These can be divided crudely into ewe and ram management factors. For both ewes and rams, management of mating to coincide with the degree of breed seasonality is essential. The general trend is for tropical or equatorial native breeds such as the Cameroon to be aseasonal and polyoestrous whereas seasonality generally increased with latitude whereby the native primitive and commercial hill breeds of Britain may only experience three oestrous cycles in a brief autumn mating period.

There are exceptions to this general relationship between latitude and seasonality, most notably the Dorset and the Romanov. The Dorset is still somewhat influenced by photoperiod: fecundity of Dorsets mated 'in season' during the typical shortening day mating period is normally higher (lambing % of 150%–175%) compared with out of season mating in which fertility and fecundity can be 20% to 30% lower.

For ewes of any breed, puberty and body weight are important limiting factors on ovarian activity. Puberty occurs early for many of the highly fecund breeds, such as the Romanov, at 3 to 4 months of age whereas 6 to 8 months of age is more typical for the majority of British or continental European breeds. If ewe lambs experience nutritional or parasitical challenges that restrict their growth rate, it is expected that puberty may be delayed in many individuals. The target of 60% to 70% of mature bodyweight is commonly used as a suitable threshold for the mating of ewe lambs as this is the point by which the majority of individuals would be expected to cycle and have also reached puberty by this time, given the expected growth rate in most sheep systems.

The management of multiparous ewes is also critical, especially the interval between weaning and mating. In sheep, locational anestrus driven by prolactin production can present problems in dairy flocks in which lactations may depress fertility and lead to ewes failing to lamb annually or in accelerated lambing protocols in which ewes are managed to lamb more frequently, most typically targeting three lamb crops in 2 years (8 month interval between lambing). In these systems a combination and interaction between both nutritional sufficiency and hormonal control reduce the fertility of ewes under these

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management systems. However, selection for ewes that are fertile during lactation has demonstrated that the trait is heritable (Cornell 'STAR' system).

The fertility and fecundity of ewes vary over the course of their life with the lowest fertility and fecundity apparent as ewe lambs, partly for reasons of variable levels of mature body weight as discussed previously. However, variation with age continues into maturity with third and fourth parity ewes demonstrating the highest fertility in most breeds and farming systems although significant variation exists between flocks due to management practices.

Oestrus Detection and Artificial Insemination

The best method of oestrus detection is with a raddled vasectomised ram, very few visible signs being detectable in the ewe. Artificial insemination (AI) in sheep has not assumed the popularity achieved in cattle; however, numbers are increasing. A number of factors have been responsible, notably the disappointing results using frozen/thawed semen deposited intravaginally or intracervically to a synchronised oestrus. The spermatozoa are unable to colonise or traverse the length of the cervix and are rapidly lost from the ewe's reproductive tract. However, lambing rates of 72% have been observed using this technique in response to natural observed oestrus as opposed to an artificial oestrus (Paulenz et al. 2005). Globally, the use of intrauterine insemination by laparoscopy has been much more successful and commonly adopted for high value breeding stock, with pregnancy rates of over 70% using both fresh extended semen and frozen–thawed semen (McKelvey 1999). Artificial insemination is best used in midoestrus or 12 to 14 hours after its onset.

Ram Effect and Teasing

Use of vasectomised or epididymectomised rams before the introduction of fertile rams is a common management practice whereby the pheromone and behavioural stimuli provided by the rams trigger ovulatory activity in the ewes resulting in a degree of synchronisation of oestrous activity. At the onset of the breeding season, when ewes are approaching the end of their anoestrous period, the use of teaser rams can allow the ewes to experience the normal first short, silent oestrus that is caused by the formation of an abnormal corpus luteum, as early as possible then allowing the first fertile oestrus to occur earlier and more synchronously for the flock than would otherwise be possible (Rosa & Bryant 2002). It has been demonstrated that 84.8% of teased ewes exhibited oestrus in the first 16 days after exposure to the fertile ram, whereas two cycles were required for the unteased ewes to show comparable activity (Smith 1991). Teasing is only effective if the ewes and rams have been isolated from any sensory contact for several weeks or months previously. The period of teasing contact is also important; once introduced to the ewes, the teaser rams should be allowed contact for 12 to 14 days before they are replaced with fertile rams.

Hormonal Manipulation of Fertility and Fecundity

Exogenous progesterone can also be used to overcome the first silent oestrous effect of abnormal anoestrous transition CL. This is most commonly supplied by intravaginal sponges impregnated with progesterone that are implanted for 12 to 14 days. They can be used in conjunction with exogenous PMSG as the most common artificial synchronisation protocol in sheep. The dose of PMSG administered varies from 100 to 900 IU, depending on the breed seasonality, the mating period, and the desired fecundity effect. Other synchronisation protocols involving GnRH and $PGF_{2\alpha}$ have also been demonstrated to be effective alternatives (Haile et al. 2016). Similar protocols are used for natural mating as for artificial insemination.

Melatonin implants are also used to advance the breeding season in moderately seasonal breeds in both rams and ewes. Melatonin implants have also been demonstrated to increase fetal numbers (Scott et al. 2009) as well as advance the breeding season.

Ram to Ewe Ratio

The number of rams per ewe will vary, depending on a number of factors: age of the ram; age of the ewes; whether more than one ram is to be used with the group of ewes; the terrain; and the size of the enclosure. Ram to ewe ratios of 1 to 25 to 1 to 40 are traditionally regarded as suitable in nonsynchronised flocks. When synchronisation is attempted, a ratio of at least 1 to 10 is commonly advised. However, far higher ratios are frequently used when rams are capable: 1 to 80 and 1 to more than 100 are not uncommon in some UK and NZ flocks. The number of rams in the mating team is also important; single sire mating groups should be avoided unless essential for parentage assignment, as transient subfertility or low libido of a single ram in single sire mating groups can present as high empty rates among ewes. It can also present as an extended lambing pattern with an unexpectedly high proportion of lambings occurring in the second or even third cycle.

Genetic Potential

Significant breed variation is observed in both fertility, fecundity, and seasonality in sheep. The modes of inheritance of the different prolificacy genes include: autosomal dominant genes with additive effects on ovulation rate (BMPR-1B; Lacaune); autosomal overdominant genes with infertility in homozygous females (GDF9); X-linked overdominant genes with infertility in homozygous females (BMP15); and X-linked maternally imprinted genes (FecX2). The size of the effect of one copy of a mutation on ovulation rate ranges from an extra 0.4 ovulations per oestrus for the FecX2 mutation to an extra 1.5 ovulations per oestrus for the BMPR-1B mutation. DNA tests enable some of these mutations to be used in genetic improvement programs based on marker assisted selection.

Noninfectious Abortions

Noninfectious abortions may be caused by rough handling (winter shearing, prehousing dipping, vaccination procedures), metabolic diseases (especially twin lamb disease), and transportation. Dog worrying is also commonly blamed for sheep abortion. Vaginal prolapse producing excessive straining, especially when infection has softened the cervical plug, may lead to abortion. Congenital goitre in lambs has also been implicated as a cause of abortion.

Noninfectious abortions rarely result in more than 2% of the flock aborting, and when the percentage is higher, it is highly likely that an infectious cause of abortion is present and should be fully investigated (see later in this chapter).

Structural Defects

Structural defects of ovine genital organs are uncommon. In a recent and extensive survey involving 33,506 ovine genital tracts

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TABLE 29.1 Frequency of isolation of pathogens from sheep fetopathies recorded by APHA and Scotland's Rural College on diagnostic submissions in Great Britain

Diagnosis	2008	2009	2010	2011	2012	2013	2014	2015
Bacillus licheniformis	1%	0%	0%	0%	0%	0%	0%	0%
Campylobacter	7%	6%	10%	7%	3%	10%	8%	7%
Chlamydia abortus	20%	19%	17%	22%	17%	14%	21%	17%
Coxiella burnetii	0%	0%	0%	0%	0%	0%	0%	0%
Dystocia	0%	1%	1%	1%	1%	1%	2%	1%
Fungi spp	0%	0%	0%	0%	0%	0%	0%	0%
Listeria spp.	2%	1%	1%	1%	1%	1%	2%	1%
Salmonella Dublin	0%	0%	1%	0%	0%	1%	0%	0%
Salmonella Montevideo	0%	0%	0%	0%	0%	0%	0%	0%
Salmonella Typhimurium	0%	0%	0%	0%	0%	0%	0%	0%
Salmonella (other spp.)	2%	2%	2%	2%	1%	2%	1%	1%
Schmallenberg	0%	0%	0%	0%	11%	2%	0%	0%
Trueperella pyogenes	1%	1%	1%	0%	1%	0%	1%	0%
Yersinia spp.	0%	0%	0%	0%	0%	0%	0%	1%
Diagnosis not reached	50%	53%	53%	55%	52%	54%	51%	53%
Other diagnosis	5%	5%	3%	3%	4%	4%	3%	4%

(9970 parous) from two UK abattoirs, Smith (1996) identified 6.57% of parous and 1.95% of nulliparous tracts with pathological lesions. Most involved the ovaries and their associated bursae, with fibrin tags and paraovarian cysts being most frequently identified. However, it is unlikely that these lesions alone would cause infertility. There is no doubt that many of the other lesions identified in this survey would have caused infertility or sterility (e.g., ovarian aplasia, ovarian hypoplasia, bilateral hydrosalpinx, aplasia of the paramesonephric ducts, freemartinism, hermaphrodism, and pseudohermaphrodism).

Owing to the rarity of anastomoses of the adjacent allantoic vessels of twins, the freemartin condition is likely to be rare, but incidences of 0.23% to 1.22% have been recorded (Smith 1996). Even higher levels have been detected in the more prolific breeds: for example, 6.85% in Booroola F ewes (Cribiu et al. 1990). True hermaphroditism has been recorded in sheep. Frayer-Hosken et al. (1992) both reported ewes with an ovotestis, whereas Smith et al. (1996) reported a ewe with one active female gonad and an inactive testis with a pampiniform plexus. Cases of intersexuality are seen mainly at lamb castration. They are male pseudohermaphrodites referred to by shepherds as 'wilgils'. The fact that several may be seen at once in a flock tends to point to a possible hereditary cause. Other developmental defects of the genital organs of sheep are rare, although there is good evidence of an association between ovarian hypoplasia and breeds with high fecundity (Davis et al. 1992, Vaughan et al. 1997).

Infectious Agents

It should be noted that all the insidious production limiting disease of sheep that lead to weight loss and ill thrift will also reduce fertility and fecundity. These include maedi visna, ovine pulmonary adenomatosis, Ovine Johnes disease, parasitical gastroenteritis, fascioliasis, haemonchosis chronic pneumonia, and caeseous lymphadenitis in advanced cases. Recent proportional distribution of abortion diagnoses (2008–2015) from sheep submissions to surveillance laboratories in the UK are summaries in Table 29.1.

Nonspecific infections of the genital tract, especially the uterus, are of minimal importance in ewes, probably because in most breeds of sheep there is a long period of anoestrus after lambing. In the small number of ewes in which bacterial contamination occurs at lambing or postpartum, which is less than 20%, they are rapidly eliminated within a week (Regassa & Noakes 1999) and thus before the genital tract can be exposed to a period of progesterone influence; this will occur at the next dioestrus, which will normally be many months away. In the cow, retention of the fetal membranes (RFM) postpartum is quite common, and this is a major risk factor in the development of endometritis and subfertility. RFM is relatively uncommon in ewes; when it does occur, attempted removal by applying traction to the exposed portions of the membranes can be attempted. If left, they will usually separate and be shed within 5 to 6 days. If an affected ewe shows signs of systemic illness due to the development of metritis, she should be treated with an appropriate broad spectrum antibiotic. However, there are a number of specific infectious agents that can have a profound effect upon fertility, particularly by causing abortion and perinatal mortality.

A number of infectious agents have a predilection for the gravid reproductive tract of sheep, frequently resulting in abortion. Abortion per se is only aspect of the phenomenon, in some instances barren ewes may be involved, as are weak nonviable lambs, either being born to time or prematurely.

All abortions occurring in a flock should be regarded as infectious unless proved otherwise.

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Those specific infectious agents that cause infertility and subfertility in sheep, especially fetopathy with abortion, will be described later in order of prevalence.

Enzootic Abortion of Ewes

Enzootic abortion of ewes (EAE), also known as ovine enzootic abortion, is consistently the most commonly diagnosed cause of infectious abortion in the UK. Infection is caused by *Chlamydia abortus* (previously classified as *Chlamydophila psittaci* immunotype 1), which has a predilection for the pregnant uterus. It may also infect goats, cattle, deer, and humans. For many years, in Scotland and the English border counties, shepherds and veterinarians were familiar with an enzootic abortion in flocks. The causal organism was identified by Stamp et al. (1950). The disease is now widespread in Britain and common in Europe and the western US but absent from Australia and New Zealand. *C. abortus* has a highly specialised life cycle involving alternate intra- and extracellular phases that confer advantages for evasion of host immune responses and facilitate the maintenance of low grade, asymptomatic infection. It occurs most commonly in flocks that are intensively managed over the parturient period.

Epidemiology

The major source of infection, responsible for over 80% of new outbreaks in clean flocks, is the purchase of infected ewes of any age (Greig 1996). Spread may also be by wildlife, e.g., foxes, gulls, and crows. Sheep-to-sheep spread is the commonest route, and lambing time is the greatest time of risk when infected ewes shed large numbers of infectious particles into the environment. Susceptible ewes inhale or ingest *C. abortus* from infected placentae and fetal fluids from aborting ewes, which are heavily contaminated and are the main source of infection for susceptible sheep (Aitken et al. 1990); dead lambs and contaminated bedding can also be implicated. Infective particles (elementary bodies) may survive for weeks at low environmental temperatures. Ewes infected early in pregnancy usually abort; otherwise the organisms lie dormant until the next pregnancy, or occasionally a subsequent pregnancy. *C. abortus* is not transmitted in the milk of infected ewes. However, lambs may acquire infection from uterine discharge on the teats. Of the lambs infected by the ewes, field evidence suggests that around 30% may develop placentitis in their first pregnancy (either as lambs or shearlings), a proportion of which may abort (Greig 1996).

Chlamydia acquired outside pregnancy lie dormant. However, they can be reactivated from their 'latent' state during pregnancy. Neither the site of latency nor the precise triggers of reactivation have been identified. Latently infected flocks are unrecognisable by immunological methods. The work of Buxton et al. (1990) demonstrated that ewes were susceptible to infection from early gestation onwards. The tonsil and lymphoid tissue of the pharynx has been shown to be a primary site of infection, with subsequent blood-borne spread to major organs and lymph nodes. Thereafter, until 60 to 90 days of pregnancy, the site of *Chlamydial* persistence has not been ascertained and, although infection of the placentae and fetuses occurred from 60 days gestation, pathological changes were not observed until after day 90. Rapid replication of *C. abortus* leads to local necrosis and contiguous spread of infection involving the cotyledonary and intercotyledonary placenta and apposing endometrium, resulting in abortion that usually occurs in the last 2 weeks of pregnancy. The macroscopic signs of a placentitis are similar to that after *Brucella abortus* infection in cattle. The intercotyledonary allantochorion is oedematous, thickened, and leathery in appearance; there is degeneration and necrosis of the fetal cotyledons and a thick yellow deposit on the chorion.

Those ewes that are infected late in pregnancy do not abort until the next pregnancy. In split lambing flocks, late lambing ewes may acquire infection from infected ewes in the earlier lambing flock and abort in the same season. Infected brought-in sheep may abort in the first year, spreading infection at lambing time to susceptible ewes and lambs, resulting in an abortion storm the next year.

Most aborted lambs are well developed, fresh, and show no autolytic changes, indicative of recent death in utero; some infected ewes may produce both dead and live lambs. However, lambs born alive may be weak, fail to survive, and, in spite of good nursing, contribute to the overall losses from EAE. A small number of ewes may develop postabortion metritis. Abortion rates vary from 5% to 30%, the upper level most likely to occur in the first or second year after the introduction of infection, and thereafter at a rate of 5% to 10%. However, these figures do not take into account lamb losses in the neonatal period, which may be as high as 25% (Greig 1996).

Although rams can become infected and may develop epididymitis, there is no evidence that they play any significant role in the transmission of EAE. In the UK, rams rarely run with ewes during lambing/abortion times and therefore are not exposed to *Chlamydial* infection.

Diagnosis

Clinical Signs. There are no premonitory signs of impending abortion. Ewes are not ill. However, a few ewes may show evidence of a vaginal discharge for several days beforehand and possibly behavioural changes. There may be abortions, premature lambs, weakly live lambs, and normal lambs with infected membranes. Ewes may retain fetal membranes leading to metritis, but no other clinical signs are seen.

Placental Lesions and Staining. The placenta is usually acutely inflamed, thickened, and necrosed showing typical signs of a placentitis (Fig. 29.1). Smears from infected intercotyledonary areas and the wet skin of the fetus can be stained by the modified Ziehl-Neelsen method to detect intracellular inclusion bodies, which occur as small, acid-fast cocci; they may be seen intracellularly as clumps or singularly scattered throughout the smear, and these may be confused with *Coxiella burnetii* organisms, which are larger.

Serology

The demonstration of specific *Chlamydia* antibody in fetal fluids or precolostral lamb serum with a fluorescent antibody test is specific evidence of *Chlamydia* infection.

The complement fixation test is the routine diagnostic test used, titres of at least 4/32 generally being accepted as positive. Paired samples should be taken at the time of abortion and 3 to 4 weeks postaborting; in positive ewes, samples show a significant rise in antibody titres. An enzyme-linked immunosorbent assay (ELISA) and indirect immunofluorescent antibody test are also available.

Treatment

Antibiotics, which will reduce rather than eliminate abortions, can be used in flocks with extended lambing seasons in the face of an epidemic outbreak in a previously uninfected flock. For the best results, treatment should be given as soon after 95 to 100 days of gestation as possible, at which time possible cases of placental

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• **Fig. 29.1** Ovine placenta infected with *C. abortus* showing evidence of acute placentitis and thickened and necrotic placentomes.

infection will have commenced. Long-acting oxytetracycline, at a dose of 20 mg/kg repeated every 10 to 14 days until lambing, has been used to arrest or retard the progress of the infection. This treatment will reduce the number of organisms shed but does not eliminate infection, nor can it reverse pathological changes already present in a heavily infected placenta; hence some abortions will still occur despite treatment. It must be emphasised that in flocks with a history of EAE or at high risk of acquiring disease through purchased replacement ewes, the routine, prophylactic antibiotic use for the control of the disease is neither appropriate nor responsible use of antibiotic agents when highly effective vaccines are available and economical.

Control

Live attenuated vaccines are very successful in preventing disease and should be regarded as the preferred means of control in flocks with endemic disease in which the early vaccination of uninfected female replacements over the generational cycle of the flock will eradicate the disease in many cases. Vaccinated ewes will have lower titres with no evidence of a rise and have occasionally been associated with abortion due to the vaccine stain potentially due to reversion to virulence; however, these cases are believed to be exceptionally rare.

Ewes that have been infected do not develop positive titres until after they have aborted, thus it is not possible to screen a flock for a latent infection. Protection by vaccination, using either Enzovac (Intervet UK) or Cevac Chlamydia (Ceva), both of which contain a temperature-sensitive strain of *C. abortus*; thus both require a 2-month time interval after vaccination before they have developed protective antibody levels. Vaccination can be used from 5 months of age, and also in older animals between 1 and 4 months pretupping. The vaccine will protect lambs from transplacental infection. High-risk flocks, i.e., those who source replacement sheep from nonaccredited flocks, should have vaccination repeated yearly or biannually. An inactive preparation, Mydiavac (Novartis UK Ltd), has also been developed, and experimental data indicates that it may reduce the level of abortion in previously infected ewes during an outbreak of infection.

Biosecurity and Biocontainment of Infection Risk

Strenuous efforts must be made to prevent infectious abortion agents from gaining entry using the following management strategies:

- Maintain a closed flock and purchase rams from known sources, or purchase replacements from EAE-monitored flocks.
- Purchased ewes should be lambed separately from the indigenous flock in the first year, and all abortions and barren ewes should be investigated.
- Isolate for up to 3 weeks, and mark all ewes that abort.
- Send further dead lambs and membranes to a laboratory for diagnosis as more than one cause of abortion may be present in the flock.
- Reduce risk of spread to other ewes.
- Dispose of dead lambs and membranes not needed for diagnosis.
- Clean lambing area and cover with clean straw.
- Discourage use of ewes to foster lambs as infection may be picked up from vaginal discharges and infected fleeces. If lambs are fostered they should not be used for breeding.

Zoonotic Risks

C. abortus from sheep can be extremely dangerous to pregnant women, growing rapidly in the unborn baby's placenta. Initial mild, influenza-like symptoms become progressively more severe, and abortion occurs within a week. Disseminated intravascular coagulation may develop in the mother, causing critical illness.

Toxoplasmosis

Toxoplasma gondii infection is the second most common cause of abortion in the UK and also in New Zealand; it has been shown to cause early embryonic mortality, abortion, stillbirths, and weakly offspring in many domestic species, including sheep, and has a worldwide distribution in animals and humans. Infection in nonpregnant sheep is typically mild and unapparent, but in pregnancy, it is essentially a disease of the conceptus and the gestational age at infection affects the nature of the disease process.

The causal organism has a complex life cycle, involving an asexual cycle that can occur in any species of mammal or bird and a sexual cycle that can only be completed in cats and wild Felidae. In the cat family, the parasite multiplies within the epithelial cells of the intestine, and as a consequence, oocysts will be excreted in the faeces for about 8 days, during which time tens of thousands of oocysts can be shed. These sporulate within a few days and are then ingested by sheep.

Epidemiology

The principal vector in the spread of toxoplasmosis is the cat and its related wild species. Infective toxoplasma oocysts may survive up to 2 years on pasture, feed, or bedding. They are passed in the faeces of young cats, which become infected when they first begin to hunt. Although toxoplasms have been demonstrated in the semen of experimentally and naturally infected rams, infection of the ewe at tupping would be unlikely to cause abortion. Toxoplasms can also be passed in milk during acute infections. Lateral spread within a flock from aborting ewes is likely to be relatively unimportant. However, lambs born alive that survive from infected ewes can be congenitally infected. As few as 200 oocysts will infect one ewe, and as many as 1 million may be present in 1 g of cat faeces. Once ingested sporozoites are released, they penetrate the intestines and are distributed to many organs, where tissue cysts form; a febrile response occurs after 5 to 12 days in conjunction with a parasitaemia. Toxoplasma can be detected in the uterine caruncular septa 10 days after oocyst ingestion and in placental trophoblast cells after 10 to 15 days; toxoplasma-specific fetal antibody is present after 30 days.

After infection, sheep are considered to remain persistently infected for life, to be immune to the parasite, and therefore unlikely

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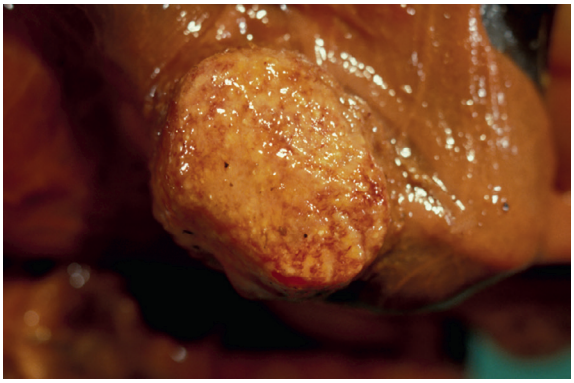
• **Fig. 29.2** Mummified ovine fetus surrounded by fetal membranes showing cotyledons associated with infection with *T. gondii*.



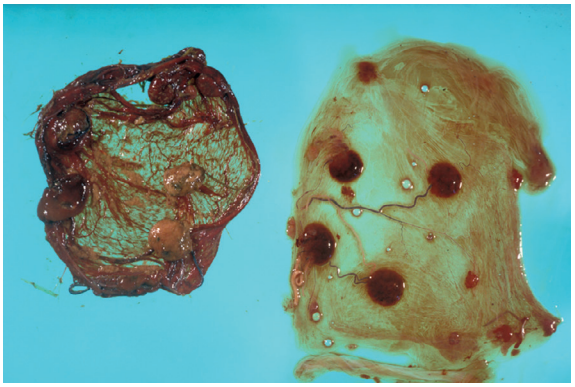
• **Fig. 29.3** Ovine cotyledon infected with *T. gondii* showing the typical 'frosted strawberry' appearance.

to abort again with toxoplasmosis (Buxton et al. 2006). However, it has been reported that a small proportion of persistently infected ewes may, in subsequent pregnancies, transmit toxoplasms to the fetus and abort; it has been suggested by Duncanson et al. (2001) that this may be due to a recrudescence in the ewe, causing a vertical transmission to the fetus. More research work is necessary to confirm this.

Clinical Signs

The effect upon reproduction depends on the stage of pregnancy when infection occurs. If early in gestation, i.e., before 60 to 70 days, fetal resorption usually occurs, with ewes returning to oestrus or remaining barren. Unlike the case with EAE, numerous barren ewes that have not been seen to abort may be detected in the flock at scanning or lambing time. With early embryonic mortality, providing a ram is still present and the breeding season has not ended, ewes are capable of conceiving, now with a good immunity. Infection in midgestation results in abortion or mummification; in the latter case, only one member of a set of twins or triplets may be involved (Fig. 29.2). Infection after 120 days usually results in stillbirth, or weakly or normal lambs.

The gross appearance of the placenta, particularly the cotyledons, is fairly typical of the disease. Cotyledons are bright to dark red with multiple small white necrotic foci 1 to 3 mm in diameter (so called 'frosted strawberries') (Fig. 29.3). These nodules may be sparse or so numerous that they become confluent; sometimes normal cotyledons are present. The intercotyledonary areas of the allantochorion appear normal (unlike infection with *C. abortus*) (Fig. 29.4).



• **Fig. 29.4** Photograph of normal allantochorion *(right)* and one infected with *T. gondii (left)*. Note that although the cotyledons appear different, the intercotyledonary allantochorion is similar in both specimens: clear and transparent.

Diagnosis

The condition is characterised by barren ewes, abortions, stillbirths, and mummified and weakly lambs. The appearance of the placenta is diagnostic.

Confirmation can be made using Giemsa- or Leishmann-stained smears of those cotyledons containing the white nodules. Alternatively, histological sections of the cotyledons may be required to demonstrate the presence of the parasite. Examination of the brain, especially in those lambs that die soon after birth, may reveal foci of glial cells and leukoencephalomalacia, which are characteristic of the infection. Immunofluorescent staining of cotyledon sections can also be used.

A number of satisfactory serological tests on the maternal serum have been used including the dye test of Sabin and Feldman, the indirect fluorescent antibody (IFA) test, radioimmunoassay, and the ELISA test. The ELISA test has been modified to detect anti-*Toxoplasma* immunoglobulin G (IgG) in body fluids. The indirect haemagglutination test (IHA) has been developed so that kits are available for use by veterinarians in practice laboratories. A single serum sample with an elevated titre may well only indicate past infection. However, serology of ewes is difficult to interpret as antibodies remain elevated for years. Paired samples may prove beneficial; when retaken after a 14-day interval, a rising titre is indicative of an active infection. Infected lambs will have precolostral antibodies, and serology can be performed on pleural, pericardial or peritoneal fetal fluids, or lamb serum. If postcolostral samples only are available, it is necessary to demonstrate IgM and IgG antibody.

Treatment and Control

Although there is virtually no danger of lateral spread from aborting ewes, isolation in the early stages of an abortion outbreak should be implemented because there is a possibility that other infectious agents may also be present.

Ewes that have aborted will be immune (see previous mention of possible recrudescence) and should be retained in the flock, and new additions should be exposed to possible infection with oocysts from contaminated food or bedding as early as possible before the start of the breeding season.

An effective vaccine (Toxovax, Intervet, UK) has been developed using living tachyzoites of the S48 strain of *T. gondii* (Buxton et al. 1991). Ewe lambs should be vaccinated from 5 months of age, and ewes and shearlings should be vaccinated during the 4-month period before tupping; pregnant animals must not be vaccinated. Recent

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studies show that the degree of protection produced by the S48 tachyzoites was as good at 18 months as 6 months after vaccination. The manufacturers state that other live vaccines should not be administered within 4 weeks of the administration of Toxovax.

Decoquinate, the anticoccidial drug fed daily at 2 mg/kg body weight, also significantly reduces the effect of *T. gondii* oocysts ingested by pregnant sheep and is licensed in the UK for this use. Both these products work best if they are being fed at the time sheep encounter infection rather than after infection has been established (Buxton et al. 1996). However, due to the nature of flock management systems and the difficulty and expense of incorporating decoquinate into a feed stuff that reliably delivers the required dose to every sheep, it is seldom if ever practical or preferable to use this approach as an alternative to vaccination.

Transmission and Pathogenesis

Without doubt, the principal vector in the spread of toxoplasmosis is the cat and its related wild species. They excrete oocysts in their faeces and contaminate pasture, forage, and other foodstuffs. Young cats and older breeding cats are the main problem. Old neutered cats on a farm are helpful, not only in reducing levels of vermin but in keeping feral cats away. Foodstuffs should be protected from faecal contamination by cats. Wild rodents, especially mice, are the main source of infection for cats.

Infection of ewes outside pregnancy produces a good immunity. Replacement ewes should be exposed to the farm environment as soon as possible after purchase so that they can ingest oocysts and acquire immunity before tupping (see previous section).

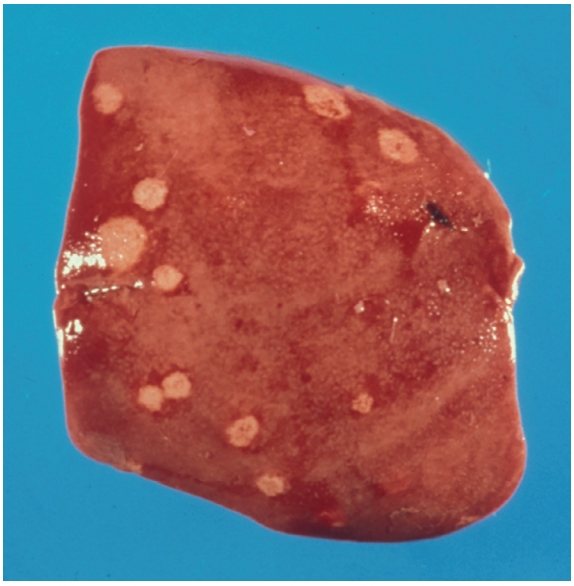
Toxoplasma gondii can affect humans but usually does not cause clinical disease. The exceptions are women who become infected for the first time during pregnancy. Placental and fetal infection results in severe damage to the unborn child. Other people at risk are those who are immunosuppressed, and they, like pregnant women, should not assist at lambing time.

Campylobacteriosis

Campylobacter is the third most common cause of abortion in the UK and the most common cause in New Zealand; both *C. fetus fetus* and *C. jejuni* cause abortion in ewes, the former being the most commonly isolated organism.

Epidemiology

Infected, and often symptomless, animals excrete these organisms in faeces. *C. jejuni* is mainly from a wildlife source and *C. fetus fetus* from carrier sheep. Unlike in cattle, in which the route of infection is mainly venereal, in sheep it is by ingestion and mainly intestinal. Once abortions occur, there is lateral spread to other susceptible pregnant ewes. The organism survives well in cold, moist conditions but soon perishes in hot, dry weather. The only clinical signs of the disease are abortion, usually in the last 6 weeks of gestation; lambs at full term may be born dead or in a weak condition. Apart from some vulvar swelling and the presence of a reddish-coloured vulvar discharge, ewes rarely show any other clinical signs. Metritis may develop after abortion, with some ewes becoming ill and even dying. Aborted material is infective. Ewes infected less than 3 months into pregnancy are not affected. If ewes are infected more than 3 months into pregnancy, a bacteraemia develops, with placentitis being the main lesion. In late pregnancy, abortion occurs between 1 and 3 weeks after infection. Flocks infected for the first time may have levels of abortion varying from 5% to 50%. There is a strong immunity after infection, but this is serotype specific. Symptomless carriers may excrete the organism for up to 18 months. In the UK, the disease dynamic appears to follow a national 4 to 7 year cycle in which campylobacteriosis alternates between being the second most commonly identified infectious abortion agent behind *C. abortus,* and low prevalence years in which it may lie fourth behind toxoplasmosis and listeriosis (VIDA surveillance data from Animal and Plant Health Agency, APHA). This pattern suggests a possible climatic effect increasing the relative risk of infection between farms as well as within a farm, dynamic susceptible population flux over time as previously infected individuals are culled and replaced with naïve individuals as part of the normal production cycle.



• **Fig. 29.5** Ovine fetal liver showing the characteristic circumscribed grey necrotic foci.

Diagnosis

- There are signs of placentitis with oedema and necrosis of the fetal cotyledons; the intercotyledonary membrane is oedematous, hyperaemic, and slightly opaque. However, these lesions are not pathognomonic. The aborted fetus looks fresh, with no specific visible gross pathology; however, in approximately 25% of the aborted fetuses, there are characteristic circumscribed, grey, necrotic foci in the liver (Fig. 29.5), 10 to 20 mm in diameter (Dennis 1991).
- Disease is most common in young ewes or older ewes that have lost their immunity.
- The organism can be identified in Gram- or modified Ziehl-Neelsen-stained smears from the placenta and fetal stomach contents and cultured from the placenta, fetal stomach, or liver.
- Serology is not useful.

Treatment and Control

- As soon as campylobacteriosis is suspected, aborting ewes should be isolated from pregnant ewes.
- Dispose of aborted materials as the disease is zoonotic. Avoid infecting sheep over 3 months in lamb or wildlife that can later act as reservoirs of infection.
- When campylobacteriosis has been confirmed, mix aborted ewes with those already lambed to stimulate production of a

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strong immunity. The disease is self-limiting, and most ewes from an infected flock acquire an immunity in the first year, irrespective of whether or not they have aborted. This acquired immunity lasts about 3 years in most circumstances, equal to the expected breeding life of the ewe.
- Turn over feed troughs, preventing birds from feeding at them.
- Try to keep the flock closed. Brought-in ewes should be mixed with the resident sheep for as long as possible before midpregnancy, then separated in late pregnancy and lambed separately.

In the US, New Zealand, Australasia, and Europe, a formalin-killed adjuvant vaccine incorporating the most prevalent serotypes (Campvax4, MSD) is commonly used. There is some evidence that, in the early stages of an outbreak of campylobacteriosis in a flock, vaccination of all remaining pregnant ewes might be worthwhile. Because there is a 10- to 14-day delay before immunity develops after vaccination, early diagnosis is imperative. In New Zealand, campylobacteriosis is the most important abortion agent; 88% of sheep farms are suspected to be infected and use of the multivalent vaccine for the four most prevalent fetus serotypes has been suggested to increase lambing rates by an average of 9%. There is considerable debate in the UK over the potential usefulness of this vaccine. This is because the serotype distribution differs from that found in NZ, abortion outbreaks are less common and flock prevalence is thought to be lower. Currently the vaccine is not licensed in the UK but is imported on special license for use in some flocks.

Listeriosis

Listeria has a worldwide distribution in several domestic species, especially ruminants, and is the fourth most common diagnosed cause of ovine abortions in the UK. Two species and multiple strains of *Listeria* produce disease in sheep, *L. monocytogenes* and *L. ivanovii,* and the disease may be presented in one or more of the following forms, relating to the strain of the bacteria involved:
- Encephalitis
- Abortion
- Diarrhoea and septicaemia
- Keratoconjunctivitis, iritis, and mastitis
- Septicaemia and death in young lambs
- Chronic mastitis

The incidence of listeriosis has increased in the UK in recent years, with encephalitis being the most common form. Neurological signs characteristic of listerial encephalitis are circling with evidence of unilateral facial paralysis, head tilting, and turning. Abortions in sheep are produced by both *L. monocytogenes* and *L. ivanovii,* and although they may occur at any stage, they are most frequent in late pregnancy. Initially, sheep may be pyrexic. There are no distinguishing characteristics to the abortion and usually no typical complications after expulsion of the fetus, which may be autolysed. Weakly lambs are often born, and grey/white miliary foci of necrosis may be seen in the fetal liver (so-called 'sawdust liver'). The placental villi are necrotic, and the chorion is covered with a brownish red exudate; there is a heavy brown vaginal discharge, and rarely death of the ewe occurs from metritis or septicaemia. It is rare for the nervous disease and abortions to occur together. Abortions under field conditions have been reported as soon as 7 days after the feeding of suspect silage.

Transmission and Pathogenesis

L. monocytogenes is ubiquitous, frequently found in soil but also isolated from foodstuffs and faeces of healthy animals. Soil is the most likely source of infection, principally after the feeding of soil-contaminated baled silage when poor fermentation conditions promote bacterial multiplication. Sheep are probably frequently exposed to infection, but presumably it requires some other factors to precipitate clinical listeriosis. After ingestion in late pregnancy, the organism penetrates the gut mucosa and infects the fetus, causing a septicaemia and placentitis, both of which may kill the fetal lamb. As a consequence, abortion occurs.

Diagnosis

Diagnosis is based on isolation of the organism from vaginal swabs, fetal membranes or the fetus, and fetal liver lesions. Fluorescent antibody techniques have also been used for diagnosis.

Treatment

Penicillin and amoxicillin are the most appropriate and commonly prescribed antibiotics for listeriosis. However, the promptness of treatment is critical in order to achieve clinical recovery in neurological cases. Abortion cases rarely show systemic signs in the ewe before the abortion, and the prevalence of disease during an outbreak seldom justifies the prophylactic treatment of the whole group or flock, unlike cases of EAE outbreaks.

Control

The incidence of listeriosis in all its forms has increased dramatically with the practice of feeding silage to sheep, especially that stored in round bales. In a survey carried out in Scotland, *L. monocytogenes* was isolated from 2.5% and 5.9% of clamp silage examined in 1983 and 1984 respectively, and from 22% and 4% samples of big bale silage. A more recent survey in Scotland showed that 10% of flocks had cases of listeriosis, and 80% of these were fed silage (Martin & Aitken 2000).

Attempts should be made during silage production to develop conditions that are not favourable for the multiplication of the organisms.
- Use additives to reduce the pH of the silage; make high-quality silage with a pH below 5, avoiding gross soil contamination (when the ash content exceeds 70 mg/kg dry matter), i.e., avoid mole hills and having forage harvesters set too low.
- Compact and seal silage the same day as made, making sure that round bales are securely tied and not punctured.
- Avoid feeding obviously mouldy or poor quality silage that can be smelled or has come from the top or sides of the pit.
- Remove any silage that has not been eaten by sheep after 48 hours.
- On farms where listeriosis occurs annually, it would be advisable not to graze any fields with livestock that are intended for silage making.

Listeriosis also has zoonotic implications.

Border Disease

This disease was first recognised in flocks along the English–Welsh border in the 1950s (Hughes et al. 1959), affecting newborn lambs that showed neurological symptoms such as tremor and a coarse fleece (so-called 'hairy shakers') and were generally weak with a high mortality rate. Since then, it has been recognised throughout the northern and southern hemisphere. It has also been shown to cause reproductive failure and may initially present as an unexpectedly high return to oestrus rate (identified by raddle marking) or as a high empty rate at ultrasound pregnancy diagnosis.

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Aetiology
The disease is due to infection with a pestivirus similar to that which causes bovine viral diarrhoea (BVD) in cattle and European swine fever.

Clinical Signs
The clinical signs in lambs are well documented elsewhere, so called 'hairy shakers' or 'fuzzy lambs' (Nettleton 1990). In adult ewes, infection results in a mild pyrexia that would probably go undiagnosed. However if ewes have been mated, the subsequent pyrexia may reduce conception rates and increase the risk of embryonic or fetal death, with subsequent mummification, abortion, or the birth of weakly lambs. Abortion can occur at any stage of gestation, although it is most common around 90 days with the voiding of a brown, mummified or swollen anasarcous fetus. The conceptus is most susceptible to experimental infection within the first 60 days of gestation, and if infection occurs at an early stage, the only clinical sign will be barrenness. The fetus becomes responsive to antigenic stimuli between Days 60 to 80 of gestation, after which other signs may become detectable in the lambs (Nettleton 1990).

Diagnosis
This can be made on clinical signs in lambs, supported by histopathological examination of the brain and spinal cord, virus isolation from the lamb, or a fluorescent antibody staining technique. Usually, there are no gross lesions detected in the placenta. Serological tests on ewes that abort or are barren have to be examined in relation to the antibody levels present in other ewes in the flock.

Transmission
The most likely source of infection is from either transiently or persistently infected individuals. The common route is via the purchases of female replacements from an infected flock that are then mixed with the existing naïve flock at or before mating, resulting in ongoing transmission during the mating/gestation period. Transiently infected individuals are those which have been infected by the virus and are viremic and infectious for a period of days or weeks before establishing a neutralising immune response and clearing the infection. Persistently infected (PI) animals are those infected *in utero* before the development of a functioning immune systems, where the virus is then misrecognised as 'self'. Thereafter, these animals never raise an antibody response to the infection and are continuously viremic. PI's are a particular risk as they continuously excrete virus although they may appear healthy (important to recognise that not all 'hairy shakers' are PI's and not all PI's are 'hairy shakers'). Although PI's are likely to have reduced fertility, they can give birth to infected progeny that themselves are a source of infection. However, PI's are generally present in only small numbers 0% to 2% of a lamb crop, so the importance of transiently infected individuals in a large group of mixed exposure can lead to 'rolling' transmission that maintains a source of infection for some indeterminate time until sufficient proportion of the flock is exposed and naturally immune to prevent further transmission. Some ram lambs can excrete the virus in their semen, and they may have poor fertility associated with small, soft testes. However, high value rams of perfectly normal appearance can be both transiently and persistently infected and should be quarantined and tested before use.

Pestiviruses from other species can also, under experimental conditions, cause border disease in sheep so that, among domestic animals – cattle in particular but also goats – represent potential sources of infection. Carlsson (1991) reported two natural outbreaks of border disease in which sheep were housed in close confinement with calves persistently infected with noncytoplasmic BVD virus.

Treatment and Control
There is no treatment, and as yet, there is no commercially available vaccine, although one may become available eventually. The disease is best controlled by ensuring that the flock remains closed, and hence the disease does not gain entry. Once it is present in a flock, it is important in the early stages of the outbreak to attempt to segregate pregnant ewes from those that have given birth to clinically affected lambs. At the same time, to ensure that nonpregnant ewes develop an immunity, they should be exposed to infection before the next mating period, and thereafter, any surviving lambs from the infected flock should not be retained for breeding and should be sent for slaughter, so that symptomless carriers do not remain.

Salmonellosis
Several serotypes cause ovine abortion, including *Salmonella abortus ovis, S. typhimurium, S. dublin,* and *S. montevideo.* Occasionally, exotic strains have been isolated, often associated with imported foreign protein.

Epidemiology
Many species of animal, including humans, may act as sources of infection, which may result in early pregnancy in barren ewes and in late pregnancy in abortions, and dead and weak lambs. Depending on the serotype, affected ewes may become ill and losses may be catastrophic in both ewes and lambs; meanwhile, those that recover may act as symptomless carriers.

Clinical Signs
These will vary with the serotype of organism and are summarised here.

Salmonella Abortus Ovis. This is a host-specific strain, once common in the southwest of England but now rarely isolated. Few systemic clinical signs occur in ewes other than abortion, which usually occurs in the last 6 weeks of pregnancy. Two distinct clinical pictures may be observed in the lambs:

- They may be born weak and die within a few hours of birth.
- They may be born healthy, suddenly become ill, and die in the first 10 days of life.

Salmonella Montevideo. This strain has been a problem in southeast Scotland for several years. Apart from abortion, there has been little evidence of systemic illness. During the severe winter of 1982, an abortion rate of 10.1% was noted in 11,500 breeding ewes. Severe weather with prolonged cold stress necessitated close congregation of these sheep for the early provision of foodstuffs. These arrangements may have compounded the problem and led to water courses being polluted, resulting in animals downstream becoming infected. However, it was eventually thought that seabirds had a major part to play. The disease is not as severe as cases of *S. typhimurium* and *S. dublin* infection, and diarrhoea is not a feature in lambs born alive (Linklater 1983).

Salmonella Typhimurium. The clinical picture in the ewe is totally different from those previously described. Anorexia, pyrexia

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(up to 41°C, or 106°F), and profuse scour are usual. A foul smelling vaginal discharge may be present. Death may occur from septicaemia or dehydration in 6 to 9 days. Lambs may be born dead, and those that are born alive may show signs of severe illness with diarrhoea and a high mortality rate. Severe losses among the ewes are not unusual. Numerous observers have commented that the disease often follows times of stress, e.g., after gathering, winter shearing, housing, and vaccination.

Salmonella Dublin. The clinical signs are not normally as severe in both ewes and lambs as with *S. typhimurium* infection. Death is usually from septicaemia or dehydration, and mortality rates are usually much lower.

Diagnosis

It is important to stress that *Salmonella* strains, other than *S. abortus ovis,* are zoonoses; hence care should be exercised when dealing with infected material.

- The clinical picture is that of scouring and pyrexic ewes. Before or in association with abortion, a foul smelling vaginal discharge may be present.
- Lambs born alive may become ill with fatal septicaemia or pneumonia, especially in cases of *S. typhimurium* and *S. dublin* infection.
- Identification of the organism follows culture of fetal stomach contents, placental tissue, or vaginal discharges. In addition, fluorescent antibody techniques may also be used for the rapid diagnosis of organisms in the same tissues.
- Serological tests can be used to diagnose infection with *S. abortus ovis.*

Transmission and Pathogenesis

S. abortus ovis is host specific and is usually introduced into a flock by infected sheep. Other strains of *Salmonella* are not host specific and can be introduced through contaminated food, water, wild birds, or other infected livestock. Once the disease has become established, it will readily spread by ingestion of contaminated bedding, food, or water. There is always a danger that symptomless carriers may remain in the flock, providing a persistent reservoir of infection.

S. montevideo appears to have a predilection for sheep and could become endemic in the sheep population of the UK. It has been isolated from mesenteric lymph nodes of sheep at abattoirs all the year round, and thus the carryover of infection from year to year can occur in sheep themselves.

Treatment and Control

A number of general principles have been suggested for the control of *S. montevideo* that are applicable to other *Salmonella* strains:

- Isolate affected sheep that have aborted, or scoured profusely, and treat with an antibiotic to which the salmonellae are sensitive, thus limiting excretion of the organism.
- Keep aborted ewes separate from those yet to lamb.
- If birds are possibly the source of infection, turn over feed troughs when not in use; regularly change the feeding area and avoid feeding on the ground.
- Avoid stress situations arising within the flock, such as frequent moving, and make sure that foodstuffs are freely available with sufficient trough space to avoid competitiveness.
- Try to prevent sheep drinking from streams and open ditches by using piped fresh water.

Brucellosis

Sheep can be infected with both *Brucella melitensis* and *B. ovis.* The former is endemic in many Mediterranean countries, Africa, and Central America; it does not occur in the UK. The latter has been reported in parts of eastern Europe, South Africa, western states of the US, New Zealand, and Australia; it does not occur in the UK.

B. melitensis is primarily a disease of sheep and goats but can affect other species, including man (Malta fever). Transmission is by direct ingestion of the products of abortion or drinking infected milk. It causes abortions, stillbirths, or weak lambs in late pregnancy. The placental lesions are similar to those identified with *B. abortus* infection in cattle, and diagnosis is by direct examination or culture of placental smears, fetal stomach contents, or vaginal discharges. Serological tests, such as the complement fixation test, are used. The disease can be controlled by using *B. melitensis* or *B. abortus* S19 vaccines.

B. ovis's main effect is upon the ram, in which it causes an epididymitis and subsequent infertility or sterility (see Chapter 36). After experimental infection of the ewe, the organism causes a placentitis followed later in gestation by abortion or the birth of small weak lambs. However, under field conditions, abortion rarely occurs. Although a ram can be infected after serving a ewe previously served by an infected ram, the ewes themselves are not infected venereally. The method of infection of ewes is not fully understood. Until recently, it was thought that *B. ovis* was host specific; however, infection has been discovered in stags showing similar clinical signs to those seen in rams. Stags contract infection from infected rams or other infected stags (Ridler 2002).

Q Fever

This is due to infection with the rickettsia, *Coxiella burnetii.* Although of little significance in sheep farming, it has been associated with a small number of outbreaks of ovine abortion in the UK, although recent seroprevalence surveillance results estimate 1.0% of sheep are seropositive, between-flock prevalence of 10.2%, and within flock prevalence of 10.2% (Scottish Government, AHDA, VIDA 2008–2015). Its main importance is its public health implications, producing influenza-like symptoms, pneumonia, and cardiac lesions in humans, and thus it is important that the agent is recognised when fetal material is examined to avoid the possibility of human infection. Microscopically, it may easily be mistaken for *B. abortus* or *C. abortus.*

Placenta and vaginal discharges are heavily contaminated, allowing spread of infection at parturition or later by aerosol from the fleece or dust in the lambing area, acting as a source for human infection.

Tick-Borne Fever

This is limited to those areas where the sheep tick *Ixodesricinus* occurs because almost all ticks harbour the infective agent *Anaplasmaphagocytophilum.* The disease is known in the southwest of England, Scotland, Scandinavia, the Netherlands, and South Africa. Abortion in adult sheep previously unexposed to ticks occurs naturally after tick-borne fever infection in late pregnancy, usually 2 to 8 days after the commencement of a fever, which can be as high as 41-42°C (107°F). A proportion of pregnant ewes may die. Some fetuses may die *in utero,* become mummified, and be expelled weeks later. Recovery in nonpregnant ewes is generally uneventful.

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Diagnosis
Diagnosis can be confirmed by identifying the organism in the leucocytes of ewes that have aborted or during the septicaemic phase.

Treatment
Oxytetracycline can be used for the rest of the naive flock in the face of an outbreak.

Control
Infected ewes that survive develop an immunity; the majority of all sheep from tick-infected areas will have acquired an immunity at an early age. Newly purchased nonacclimatised sheep should be introduced to farms before tupping, preferably when tick numbers are at their highest. Ticks can also be controlled by adopting the appropriate dipping routine.

It is noteworthy that rams during the breeding season, when exposed to tick-borne fever for the first time, can have reduced fertility for several months due to poor sperm quality.

Bluetongue
The bluetongue virus (BTV) appears to have originated on the African continent, where it had adapted itself to both vertebrate and invertebrate hosts, and for many years it was accepted that it was confined to the central African continent (Bruere & West 1993). However, spread occurred to the north African countries surrounding the Mediterranean and into Cyprus and Turkey in the mid-1940s. Bluetongue was first diagnosed in Texas in 1948, and by 1960 it was present in 11 of the states of the US, where it was detected in cattle, sheep, goats, and wild ruminants (Bruere & West 1993).

It was first recognised in Europe in 1956, being seen in both sheep and cattle first in Portugal and then Spain. In the late summer and autumn of 2006, outbreaks of bluetongue in sheep and cattle occurred in northwest Europe. A total of 2056 cases were reported up to January, 2007; in September, 2007, the disease was reported for the first time in the UK. Bluetongue is caused by the bluetongue virus (BTV), which is transmitted by the *Culicoides* midge; it affects all ruminants, especially sheep, in which the infection can be serious, as in some wild ruminants, e.g., whitetail deer. The northern European outbreak was caused by bluetongue virus serotype 8 (BTV-8), not previously detected in Europe, and the vector was the midge *Culicoidesdewulfi.* Interestingly, some European breeds of sheep are more susceptible to infection than other breeds. The disease has been seen in the Mediterranean countries of Europe for some time where the climate is warmer, and the vector midge is able to overwinter or the virus survives longer in the susceptible host (Elliott 2007). The most serious clinical signs and reproductive losses were experienced in Greece and the Balkans due to BTV-4.

Epidemiology
This is about the relationship between the bluetongue virus, the vector (the midge), and the ruminant host, the interaction and outcome of which is dependent on climatic factors, especially temperature. Bluetongue does not spread by direct contact and is only spread by certain species of the biting midge (Elliott 2007). It is also possible that the virus may be transmitted by using the same needle on a flock of sheep during vaccination procedures. Cattle are important in the epidemiology of the disease, the main reservoir of infection, and may remain viraemic for up to 100 days.

Clinical
Signs are due to widespread damage of the endothelium of blood vessels; pyrexia occurs, lameness due to haemorrhages in the coronary band, and salivation due to hyperaemia of the muzzle, tongue, and buccal mucosa; purple-blue discoloration of the tongue may be seen, hence the disease name of bluetongue. Mortality rates are generally low – less than 5% – although very rare instances of high mortality higher than 50% have been reported. Morbidity is high, with transient period of pyrexia, inappetance, and lethargy, Poor conception rate may be seen, and reproductive losses may occur in pregnant sheep with early embryonic mortality, abortion, mummification, and the birth of stillborn or deformed lambs that may be blind and unable to stand. Milk yield is reduced; rams may be temporarily infertile.

No specific treatment is available, and it has to be symptomatic. Immunity after natural infection appears to be enduring but serotype specific, with little cross protection against other serotypes.

Diagnosis
Various serological tests are available to demonstrate antibodies to the virus; the virus can also be isolated from blood or lymphoid tissues.

Control
Serotype-specific vaccines have been developed, and their use in sheep has reduced morbidity and mortality.

Schmallenberg
In 2011 a novel orthobunyavirus, provisionally named Schmallenberg virus (SBV), was identified in Germany affecting cattle and shortly afterwards associated with an epizootic outbreak of congenital malformations, featuring an arthrogryposis hydranencephaly syndrome, in newborn lambs. In areas that reported congenital malformations due to SBV in France, a within-flock seroprevalence of 30% was detected. Between clinically affected and not clinically affected flocks, no significant differences in seroprevalences were found. Variable seroprevalence was confirmed in most European countries over the next 2 years reaching as high as 89% in the Netherlands. Transmission was via a range of midge hosts whose activity was highest during the warmer summer months, leading to the highest proportion of clinical signs being associated with early lambing flocks that were mated during the high transmission period. Late lambing flocks were exposed and derived some degree of protective immunity during this period and were not as susceptible to disease during their own subsequent pregnancy. However, data from the UK confirmed ongoing transmission throughout the winter (Davies et al. 2013), indicating that there was no true vector-free period, and reemergence of the virus during the autumn in a susceptible population could result in more widespread disease in the bulk of the national sheep flock. Wider reproductive effects of infection were investigated by Luttikholt et al. (2014), who identified an increased return to oestrous rate in infected flocks but no other significant reproductive effects such as abortion rate. The weaning rate of lambs per ewe encompasses all causes of reproductive and postnatal–lactational period losses, a 10% reduction associated with SBV diagnosis at a flock level was reported in Ireland during the epidemic outbreak (Barrett et al. 2015).

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Goats

In the absence of any major infectious cause of abortion, infertility in the goat is generally not a major problem, normally with only a small number of barren does remaining at the end of the breeding season.

Structural Defects

Abnormal sexual differentiation during embryological and fetal development, resulting in intersexes, is relatively common in the goat, especially in breeds such as the Alpine, Saanen, and Toggenburg. It is much more prevalent in polled individuals, in which 'polledness' is a simple dominant character with full penetrance but is also associated with a recessive hermaphrodite effect with incomplete penetrance. Intersexes can also occur as a result of freemartinism, in which placental fusion occurs in twins of dissimilar sex; examples of goat freemartinism have been reported. However, the incidence of hermaphroditism appears to be higher than that of freemartinism in this species; freemartinism in goats is much less common than in cattle (Jackson 2004).

Hermaphrodites that have been described in goats are mainly male pseudohermaphrodites, having testes and the accessory reproductive organs of the female; they are genetic females.

Intersexes vary in the degree of external structural abnormality. Most are generally female-like in appearance at birth, but as they grow and mature, there will be evidence of an enlarged clitoris, perhaps testes in the inguinal region, and the development of male secondary sex characteristics, including the typical male odour (see Chapter 9).

Functional Factors

The goat is a seasonal breeder responding to the effects of declining day length. It is not unusual to have irregular oestrous cycles at the beginning and end of the breeding season, especially in goatlings, with short cycles of between 5 and 7 days.

Anoestrus may be due to starvation, parasitism, or mineral deficiencies. In the case of the latter, phosphorus and the trace elements copper, iodine, and manganese as well as vitamin E have been implicated. It can also be influenced by chronic debilitating diseases.

Hydrometra or Pseudopregnancy ('Cloudburst')

Hydrometra is the accumulation of sterile secretions within the uterine lumen. The aetiology of the condition is not known precisely, but it is always associated with high progesterone levels secreted by a persistent functional corpus luteum (CL), cessation of cyclical activity, and variable degrees of abdominal distension. The incidence of the disease varies between herds and within the same herd from year to year. Studies involving 71 dairy herds in France found an overall incidence of 2% to 3% (Mialot et al. 1991), although on one farm in the study it was 20%. In Holland, Hesselink (1993a) found a mean incidence in three herds, totalling 550 does, of 9%. The disease occurs more frequently in older does and is uncommon in yearlings. A more recent study in the UK (Griffiths et al. 2006) detected an overall incidence of 5.74% (511 cases out of 8906 does). Other findings were similar to those of Hesselink, the incidence in maiden females (0.94%) being much less than the incidence in adults (6.64%).

In Hesselink's study (1993b), there also appeared to be an association with the use of progestogen sponges and eCG treatment to advance the onset of cyclical activity before the start of the normal breeding season.

One possible cause of hydrometra is early embryonic loss. However, not all animals will have been mated by the buck before the development of pseudopregnancy.

Two types of pseudopregnancy occur:

- Fertilisation after mating is followed by early embryonic death, the CL persists, and the doe acts as if pregnant. The abdomen becomes enlarged, and in some there is a degree of udder development if not the onset of lactogenesis. Those that are lactating may have a fall in yield. This type of false pregnancy generally lasts for the duration of the gestation period or even longer, until the CL has regressed spontaneously. The term 'cloudburst' is used to describe the voiding of large volumes of fluid from the uterus as the pseudopregnancy is terminated. After this, the abdominal distension disappears; some does may search for the 'missing' kids.
- After oestrus, when the doe was not mated, there is cessation of cyclical ovarian activity, but there is no marked hydrometra. At the end of the period of acyclicity, affected does expel a bloody discharge. Therefore any unbred does that do not return to oestrus after their first oestrous cycle in the autumn should be treated with $PGF_{2\alpha}$ for possible pseudopregnancy.

Differentiation of hydrometra from normal pregnancy can be made using transabdominal B-mode ultrasound imaging, demonstrating the presence of a fluid-filled uterus in the absence of a fetus or placentomes. After 50 days of anoestrus, pregnancy and pseudopregnancy can be differentiated on the basis of serum oestronesulphate levels (see pregnancy diagnosis in goats in Chapter 5). Treatment with 2.5 mg $PGF_{2\alpha}$ will be followed by expulsion of the fluid, and oestrus will occur in approximately 4 days. By using a second injection 12 days after the first, good levels of fertility can be achieved with 85% conceiving, compared with 95% of unaffected animals (Hesselink 1993b).

Cystic Ovarian Disease

Lyngset (1968) found an incidence of 2.4% ovarian cysts at necropsy. Most were unilateral, single, and varied in size between 1.2 and 3.7 cm. Jackson (2004) quotes an abattoir survey in which cysts were seen in 12% of does examined, but the nature of the cysts and their progesterone contents were not known.

Cystic ovarian disease has been described in dairy breeds and has been particularly evident where they have grazed oestrogenic clovers and legumes. A history of nymphomania may suggest follicular degeneration, and the typical clinical signs are those of continuous oestrous and short interoestrous intervals with a failure to conceive. They should be treated with 1500 to 2500 IU of human chorionic gonadotrophin (hCG), gonadotrophin-releasing hormone (GnRH), or progesterone treatment for 18 days.

Abortion in Angora Goats

Abortion has been recognised as a problem in Angora goats in the Eastern Cape province of South Africa since the turn of the century, and it has reached such proportions as to threaten the viability of the mohair industry.

Recent studies have identified two types of abortion:

- The most common is a 'stress-induced' abortion, which occurs in poorly grown and immature does. The abortus is fresh and

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TABLE 29.2 Frequency of isolation of pathogens from sheep fetopathies recorded by APHA and Scotland's Rural College on diagnostic submissions in Great Britain

Abortion Diagnosis	2008	2009	2010	2011	2012	2013	2014	2015
Bacillus licheniformis	0%	0%	3%	0%	0%	0%	0%	2%
Campylobacter not otherwise specified	3%	4%	0%	0%	0%	0%	0%	2%
Chlamydia abortus	22%	20%	15%	4%	7%	7%	2%	0%
Coxiella burnetii	3%	4%	6%	0%	7%	0%	5%	7%
Listeria spp.	5%	0%	3%	4%	0%	2%	7%	2%
Toxoplasmosis	0%	4%	3%	4%	2%	2%	17%	8%
Trueperella pyogenes	3%	0%	0%	0%	0%	0%	0%	0%
Schmallenberg	0%	0%	0%	0%	4%	0%	0%	0%
Yersinia spp.	0%	4%	0%	0%	0%	0%	0%	0%
Other diagnosis	0%	8%	0%	4%	4%	0%	0%	3%
Diagnosis not reached	65%	56%	71%	86%	76%	89%	68%	77%

may be born alive. This is the major type of abortion loss in production operations. High metabolic requirements for fibre production in the Angora predispose the breed to nutritional and other forms of stress to a greater degree than goats of other breeds. It has been suggested that hypoglycaemia in pregnant does stimulates the immature fetal adrenal to produce oestrogen precursors, which result in the placental synthesis of oestrogens and subsequent abortions; blood glucose concentrations are influenced by short-term interruptions in food intake. Most abortions occur between 90 and 120 days of gestation, at the time of rapid fetal growth. It may be seen as a storm 1 to 2 days after stress.

- 'Habitual abortion' is probably a genetically determined hyperactivity of the maternal adrenal cortex, prematurely initiating the process of parturition. Fetuses, which are usually born dead, are often oedematous. Habitual aborters should be culled, together with any live offspring that may have survived. Does abort in the absence of stress, and levels as high as 5% may occur in flocks that do not practise culling.

Management Factors

Timing of Service or Artificial Insemination

Optimum pregnancy rates are obtained when does are mated towards the end of oestrus (which lasts 12–36 hours) and just before ovulation. Some goat owners serve their does only in the first 12 hours of oestrus, supposedly to increase the number of female kids but with a consequential reduction in pregnancy rates.

Nutrition

Vitamin A, certain minerals (manganese and iodine), and energy deficiencies reduce fertility and may be associated with abortion when the deficiency is chronic.

Stress

Stress-induced abortion has been described previously in Angora goats. However, there is evidence that other breeds of goat will abort if subjected to stress. This can result from being chased by dogs, inadequate feeding, transportation, and adverse weather, particularly during the fourth month of gestation.

Infectious Agents

Nonspecific infections appear to play a minor role in causing infertility in does, probably for similar reasons discussed previously for the ewe. However, specific infectious agents are important in causing abortion. Unfortunately, little information on their relative importance in the UK is available. Many of these specific infectious agents are also important in sheep. A survey of diagnoses recorded at VLA Regional Laboratories in England and Wales and Disease Surveillance Centres in Scotland listing the infectious causes of goat fetopathy identified in material submitted to their laboratories from 2008 to 2015 is summarised in Table 29.2.

Brucellosis

Brucella melitensis is the organism most frequently involved and is endemic in many Mediterranean countries, Africa, and Central America; it does not occur in the UK. *B. abortus* has occasionally caused abortion, but *B. ovis* has not been isolated from goats.

B. melitensis causes abortion in late pregnancy, stillbirths, or weakly kids; after the first exposure, abortion may be in the form of a storm. Few abortions occur in other years, and some does may become sterile from uterine lesions. Kids may become infected from does' milk. The disease can be diagnosed by bacterial culture from the fetus, fetal membranes, or vaginal discharges and can be controlled by routine vaccination. It is important to remember that *B. melitensis* is a zoonosis.

Campylobacteriosis

This is uncommon. It is caused by infection with *Campylobacter spp.,* probably *C. jejuni* and possibly *C. fetus fetus.* Does may or may not show evidence of a systemic illness and may abort in late gestation or produce stillborn or weakly kids and have a postabortion muco- or sanguinopurulent discharge. Multiple

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necrotic foci up to 2 mm in diameter may be seen in the liver of the aborted kids. Diagnosis, treatment, and control are similar to those described for sheep.

Chlamydophila *(Enzootic) Abortion*

This is an important cause of infertility in goats in many countries and is the most common cause of infectious goat abortion in the US. Infection is due to *Chlamydophila abortus,* which is similar to, or identical with, the strain responsible for enzootic abortion in sheep.

Abortions usually occur in the last 4 weeks of gestation, with levels as high as 25% to 60% in does kidding for the first time; stillborn and weakly kids can also occur.

Mathews (2007) states that a number of features different from those seen in sheep may be seen in goats:

- Abortion may occur at any time of pregnancy.
- Colonisation of the placenta may occur at any stage of pregnancy.
- From time of infection to abortion may be as little as 2 weeks.
- Vaginal secretions may occur as early as 9 days before abortion and last as long as 12 days after abortion.
- The doe may become ill with septicaemia and pneumonia postabortion.

Diagnosis and treatment are similar to those described previously for sheep. Does infected in late pregnancy usually abort during the subsequent pregnancy and can produce infected kids, which, after a latent phase, abort during their first pregnancy. The disease is best controlled by good hygiene to prevent lateral spread to susceptible animals, especially kids and young does, and the use of a vaccine, which has been made compulsory in some countries where the disease has become widespread. Enzovax, which is used in sheep, is not licensed for use in goats in the UK. *C. abortus* is a zoonosis.

Listeriosis

Encephalitis due to *Listeria monocytogenes* infection is quite common in goats. The same organism can cause abortion in late gestation or stillbirth. The does may show no signs before aborting but may develop a necrotic metritis and vaginal discharge shortly afterwards.

As in sheep and cattle, poorly fermented, soil-contaminated silage is a likely source of infection. The pathogenesis, diagnosis, treatment, and control are similar to those described for sheep.

Salmonellosis

There are no host-specific *Salmonella* species in the goat. However, the ubiquitous salmonellae have been reported to cause abortion.

Toxoplasmosis

Toxoplasma gondii is the most common cause of infectious goat abortion in the UK. It causes fetal death with resorption if infection occurs early in gestation, or abortion of kids, which may be stillborn, alive but weak, or normal, depending on the time in pregnancy that the doe was exposed to infection. However, unlike in sheep, fetal death is preceded in some cases by a period of severe illness with pyrexia, anorexia, diarrhoea, and muscle weakness. During an acute infection, toxoplasms may be excreted in the milk and be a source of human infection if the milk is drunk unpasteurised (Skinner et al. 1990). Also, experimentally at least, toxoplasms may be present in goat semen for a variable time after infection, but the epidemiological significance of this, as in sheep, may be very slight.

The placental lesions are very similar to those described in sheep. Diagnosis is dependent on identification of the organism in placental tissue or serological tests. Buxton (1998) states that, similar to sheep, the majority of does develop a protective immunity after infection and are protected against future challenges during pregnancy and should be retained within the herd, whereas young, nonpregnant does should be exposed to infection before they become pregnant. However, repeated abortion has been reported. Harwood (2007) states 'that immunity in goats following infection appears to be poor, or short lived, in comparison to sheep, and as a result some goats that have been previously exposed and considered to be immune may be susceptible to a new environmental challenge or to the reactivation of an existing latent infection during times of stress, disease, or injury'. (See Recrudescence under Toxoplasmosis in sheep (p. 519).)

Domestic cats and wild Felidae play a critical role in the spread of the disease, as in sheep.

Treatment and control measures are similar to those described for sheep (see previous mention). Toxovax is not licensed for use in goats.

T. gondii is a zoonosis and thus care should be taken in handling possibly infected material.

Border Disease

Border disease is only rarely recorded in goats in the UK. There appears to be only one single case of natural disease quoted in the literature, which occurred in Norway; however, Harwood (2007) states that two confirmed cases were investigated by the VLA, both of which had direct contact with known infected sheep.

After experimental infection, the disease presents a different clinical picture from that seen in sheep (Harwood 2007); early abortion, fetal resorption, and mummification occur more commonly whereas the delivery of weak, full-term kids or kids with tremors occurs less commonly. Marked placentitis is another notable feature.

Q Fever

Q fever, caused by *Coxiella burnetii,* can cause abortion and stillbirth in goats without previous clinical signs or after a few days of illness involving dullness, depression, and anorexia. The abortion rate can be very high in some infected herds (5%–50%). Large numbers of organisms are expelled into the environment from placental tissue, uterine fluids, colostrum, and milk (see Sheep section). Ticks have also been implicated in the spread of Q fever and may be the initial means of introduction into the herd. Between 2007 and 2010 a large scale zoonotic outbreak of Q fever led to over 4000 human cases. The most important risk factor for human Q fever was living close (< 5 km) to an infected dairy goat farm (van der Hoek et al. 2012). Occupational exposure of farmers and workers played a much smaller role. In 2009 several veterinary control measures were implemented, including mandatory vaccination of dairy goats and dairy sheep, improved hygiene measures, and culling of pregnant animals on infected farms (van der Hoek et al. 2012).

Diagnosis is made upon identification of the organism in smears of the placenta or the organs of the abortus and serological tests demonstrating a rising antibody titre.

Vaccination is possible but does infected before vaccination can remain chronic carriers of the organism; *C. burnetii* is a zoonosis and is excreted in milk.

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30

Management of Breeding in Small Poultry Production Units

MICHAEL IAN CLARK

With the growing trend for keeping backyard/hobby poultry, it can be a daunting task for the new graduate to feel adequately equipped to deal with the veterinary requirement of owners. However, if new graduates draw together their anatomical, physiological, pathological, and medical knowledge of avian reproduction, then, subsequently, there is much that they can offer a client, whether there is a fertility or an egg production issue in a small flock.

This chapter will deal purely with the backyard chicken (Fig. 30.1), *Gallus gallus domesticus*. Though much that will be discussed will be pertinent to other kept poultry (turkey and waterfowl) and gamebirds (pheasant and partridge), there is some fundamental species variation.

Backyard poultry tend to be kept extensively, with the main purposes of companionship, egg production for human consumption, and occasionally breeding of replacement birds. Primarily, owners source their hens from pullet rearers or acquire former commercial laying hens. A female chicken is a 'pullet' once she is no longer a 'chick' (i.e., she has lost her feathery chick down and acquired adult feathering) but has not reached sexual maturity and laid an egg. Point-of-lay pullets are sold between 16 to 18 weeks of age, with the expectation that they will come into lay in the following month. The onset of lay is governed by three factors: the pullet being sufficiently old, being in adequate body condition, and subject to the correct light stimulation. There is a huge variation in the size of pullet rearing enterprises with many different breeds being reared, ranging from small silkies to the much larger Rhode Island Red. Former commercial hens will be acquired at the end of their commercial viability, which is typically 72 weeks of age. The four commercial systems used in the UK are: enriched cages, barn production, free range, and organic free range. Production can range from between 300 and 340 eggs per hen with the more intensive systems, resulting in the highest production figures. The commercial breeds commonly seen include Hyline, Lohmann, Bovan, and Novogen. The primary breed companies that produce these birds are a very good source of information on their husbandry and maximising their genetic reproductive potential (e.g., Hyline Website 2016, Lohmann Website 2011).

If there is no requirement for the backyard flock to produce progeny, then it is not necessary to have a cockerel. In fact, they are often perceived as a nuisance due to their early morning crowing and aggressive/protective behaviour. Many owners do put up with these inconveniences and have a cockerel in the flock, as there is a perception that this maintains a more natural flock dynamic. If fertilisation of eggs does occur but there is no incubation either naturally with broody hens or artificially with incubators, then there is no embryonic development, and the eggs are fit for human consumption. A few backyard flock keepers have birds that are intended to produce fertile eggs for incubation and chick production. For the amateur enthusiast, this can be frustrating because veterinary intervention is required to investigate any combination of low egg production, low fertility, low hatchability, and poor chick viability.

Anatomy of the Female Reproductive System

In the majority of avian species, only the left ovary is functional, and this holds true for chickens. Although the right one is present embryologically, it regresses during development and is vestigial in the adult bird. If the functional ovary is removed surgically or is destroyed by disease, the right rudiment enlarges and becomes functional. The age at which the removal of the functional ovary occurs determines the future development of the rudiment. If the ovary is removed from chickens younger than 20 days old, the rudiment hypertrophies into a structure resembling a testis and is capable of spermatogenesis. However, because the Wolffian duct system does not develop in genetic females, there is no duct connection between the testis and the copulatory organ in the cloaca. The structure of the normal poultry reproductive tract is shown in Fig. 30.2.

Ovary

Immature left ovaries are amorphous, granular, grey, triangular structures ventral to the cranial lobe of the left kidney. An active left ovary can take up a significant part of the mid coelom. Numerous developing follicles can be seen ranging from the smallest white pin head sized to the most mature follicles that are the size of egg yolks (as that is what they become) and are a rich yellowy/orange in colour. This is a single cell, and the yolk represents a very large lipid inclusion in its cytoplasm.

On hatching, a female chick has millions of oocytes, most of which become atretic. The remaining few follicles slowly grow for months or years. Neutral lipids are deposited as yolk in the

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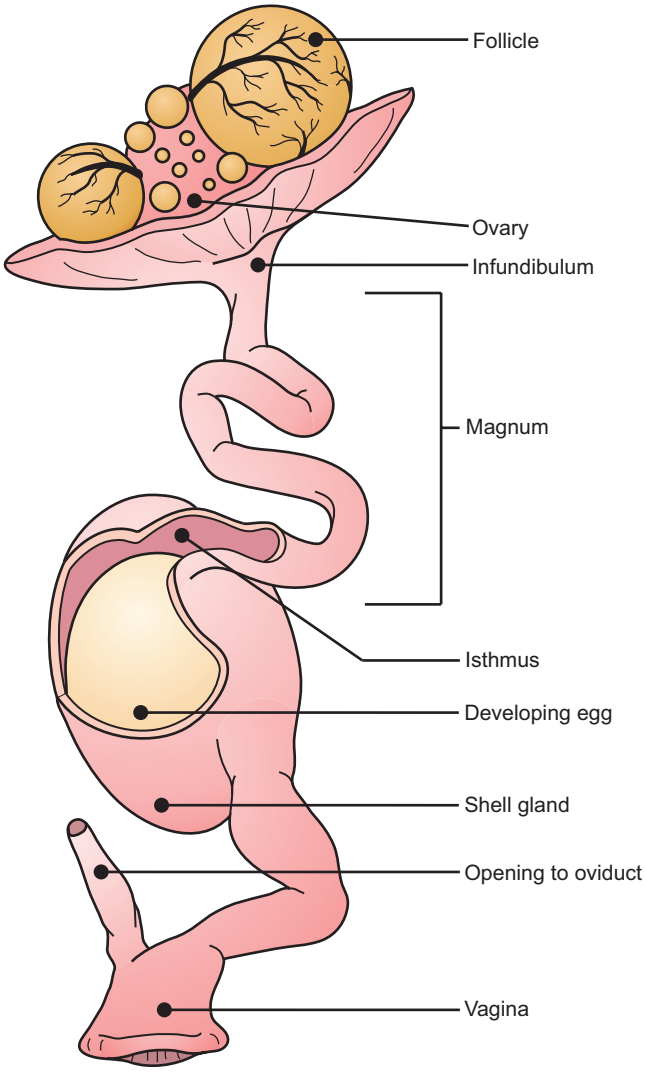
• **Fig. 30.1** Backyard chickens: *Gallus gallus domesticus*.



• **Fig. 30.2** A diagram of the female poultry reproductive tract.

oocytes, 8 to 9 weeks before onset of lay; then a small number oocytes start to enlarge, depositing more white, primordial yolk. They then enter the final rapid growth phase, with deposition of yellow yolk, which has lipids along with water, proteins, minerals, and vitamins. Follicles grow to larger than 35 mm in 7 to 11 days in the domestic hen. Developing follicles are an oocyte, surrounded by granulosa cells, and an outer layer of thecal cells. There is no fluid-filled cavity; the entire follicle is filled with yolk material. In the avian ovary, follicles do not mature synchronously; there is a hierarchy of maturation with the largest most orange coloured follicle being the next to ovulate. The next largest follicle will ovulate 25 hours later, and so on. Usually a hen's ovary has up to 10 follicles containing yolk, a larger number of small yellow follicles, and numerous small white follicles awaiting recruitment or atresia.

Oviduct

Anatomically, the oviduct is relatively complicated as it is not just involved in transporting the ovum. Spermatozoa enter the oviduct and are either stored or ushered up the oviduct to fertilise the ovum. The fertilised ovum is enveloped by the nutritious albumin and numerous membranes added before it being encapsulated by the protective shell as it journeys through the oviduct to the cloaca. The oviduct has several components: the infundibulum, magnum, isthmus, shell gland, and vagina. In immature or out of lay hens, the oviduct is a relatively small structure, but in a sexually active hen, it can be a 60 cm coil of glandular tissue of varying diameters traveling on a circuitous route from the left ovary through the left, dorsal, caudal body cavity to the cloaca.

Infundibulum

As the follicle is released from the ovary, it has to be captured by the infundibulum. The infundibulum is attached to the body wall and closely associated with the left abdominal air sac. The fluted part to the infundibulum grasps the oocyte before its passing through the infundibulum in 15 minutes.

Magnum

The magnum is highly coiled and the longest part of the oviduct. There are massive mucosal folds that add about half the egg's total albumin during its 3-hour passage through the magnum.

Isthmus

After a narrow aglandular zone, there is the isthmus that is thinner and has fewer mucosal folds than the magnum; further albumin is added and the two subshell membranes are applied to the egg during its 1-hour passage.

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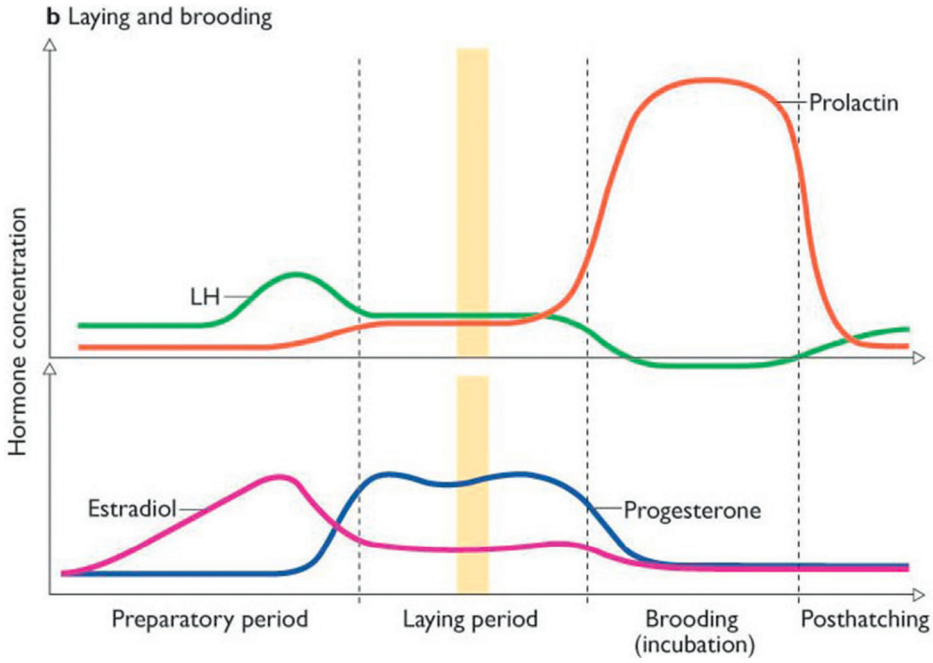

• **Fig. 30.3** Hen Egg Laying Endocrinology The four hormones – luteinising hormone, prolactin, oestradiol, and progesterone – are graphed through the four phases – preparatory period, laying period, brooding, and posthatching. (From Sjaastad OV, Sand O, Hove K, *Physiology of Domestic Animals*, 2003. Scandinavian Veterinary Press. http://www.scanvetpress.com.)

Shell Gland

During the egg's 20-hour presence in the shell gland (uterus), further albumin is added from the flattened mucosal cells. There is deposition of the shell, its glaze, and the outer cuticle.

Vagina

The egg passes through the vagina in a few seconds. The vagina ends at a slit-like opening in the lateral wall of the middle chamber of the cloaca (urodeum). When the egg is laid (air cell end first), the vaginal opening protrudes through the vent, minimising contact with faeces (Dyce et al. 2002).

Female Reproductive Physiology

There are major endocrine differences in the reproductive cycle of avian species compared with mammals. For example, in birds, granulosa cells (cells surrounding the vesicular ovarian follicle) produce progesterone (rather than oestradiol as occurs in mammals). Most of this diffuses to thecal cells (cells making up the envelope of condensed connective tissue surrounding the ovarian follicle) and is converted to oestrogen.

Fig. 30.3 illustrates the hormonal changes (for luteinising hormone (LH), prolactin, oestradiol, and progesterone) encountered by a hen through the four phases:

- Preparatory period
- Laying period
- Brooding
- Posthatching

As with any endocrine cycle, it is difficult to conceptualise a starting point. However, it is sensible to focus initially on the rising oestradiol levels in preparation for the onset of lay:

1. Synthesis of calcium ATPase in the shell gland;
2. Changes in bone composition – 10 days before egg formation, calcify medullary spaces of long bones, especially tibia and femur; this will mobilise calcium for use in shell formation;
3. Production of very low density lipid protein (VLDL) by the liver, which is a major precursor of yellow yolk, and is transported to the ovary and deposited in yolk;
4. Increased size of the oviduct (important for egg formation); and
5. Together with male sex hormones causes changes to plumage, comb size, sexual receptivity to males

As follicles mature, enzyme activity decreases in the theca cells but increases in the granulosa cells, resulting in a shift to increased progesterone synthesis near the time of ovulation. This can be seen in the rising blue line just before laying in Fig. 30.4.

The ovulation positive feedback loop that occurs in poultry (Fig. 30.5), superficially, may look similar to that of mammals; however, there are differences:

- It is progesterone, not oestrogen that is involved in this positive feedback loop.
- Ovulation of the dominant follicle (given the nomenclature F1) in birds is preceded by an increase in progesterone and LH.
- LH stimulates secretion of more progesterone from the granulosa layer of the mature ovulatory follicle.
- In experiments, administration of exogenous progesterone results in an LH surge and ovulation, so many believe it is progesterone that triggers the LH surge, not oestrogen as it does in mammals.

In both mammalian and avian species, LH and oestradiol increase before ovulation. The big difference is that in mammals, progesterone only increases after ovulation, whereas in birds, progesterone

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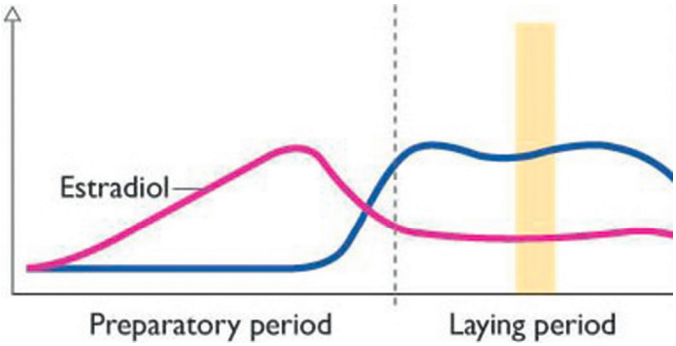
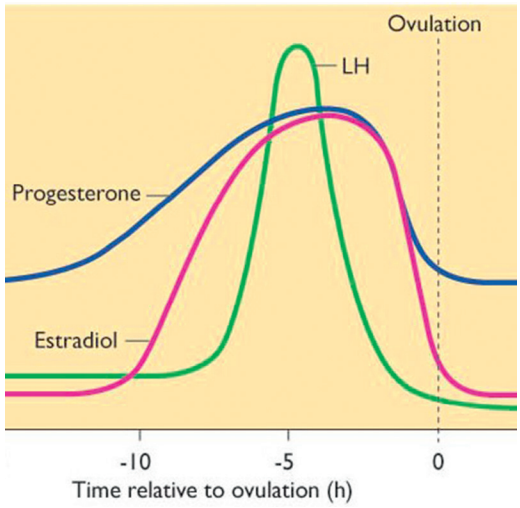
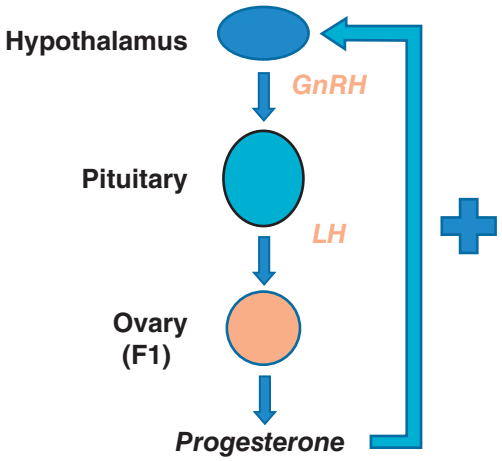


• **Fig. 30.4** Oestradiol and progesterone changes during reproductive phases in the laying hen. (From Sjaastad OV, Sand O, Hove K, *Physiology of Domestic Animals,* 2003. © Scandinavian Veterinary Press. http://www.scanvetpress.com.)



• **Fig. 30.6** Avian hormone changes around the time of ovulation.



• **Fig. 30.5** The avian ovulation positive feedback loop. N.B. it is progesterone that is involved with this loop. (With the permission of Yon L. Avian Reproduction. Unpublished lecture notes, 2017.)

increases before ovulation (Fig. 30.6) and is actually responsible for the LH surge which triggers ovulation.

There is an 'open period', which is a window of time each day when LH release can occur. It is usually 8 to 10 hours long and is from the onset of darkness to about 1 hour after the onset of light. Dusk signals the hypothalamus to set the circadian clock, which then sets the timing of the open period.

If the largest follicle (F1) produces and releases enough progesterone during the open period, then an LH surge and ovulation occur. After ovulation, unlike in mammals, no corpus luteum is formed in birds as there is no requirement to sustain a pregnancy. Synthesis of progesterone is restricted to the few mature follicles that are present because progesterone is responsible for the positive feedback cycle that triggers LH surge and ovulation. If it is too high, it will suppress GnRH and LH.

In domestic hens, ovulation occurs 6 to 8 hours after the LH surge, and then approximately 25 to 26 hours after ovulation, the egg is laid. Ovulation usually occurs 15 to 75 minutes after oviposition (time of lay is a practical guide to time of ovulation). Each ovulation occurs slightly later on subsequent days; as a consequence the hen eventually 'misses' the open period for that day's ovulation, resulting in a pause of 1 day in egg production.

Male Reproductive Anatomy

Testes

Chicken testes (left and right) can be seen during embryonic development at the cranial poles of the kidneys. Unlike mammals, there is no migration from this position during sexual maturation. Before sexual maturity the testes resemble grains of rice. In sexually active cockerels the testes are large white structures up to 5 cm long; in the quiescent period, they shrink to about half that size (Fig. 30.7). The seminiferous epithelium contains developing germ cells in distinct associations referred to as 'stages'. The stages are arranged sequentially in a helix that extends along the length of the seminiferous tubule (Scanes 2015). There are no accessory sex glands in cockerels, resulting in a modest ejaculate of approximately 1 ml of semen.

Epididymis and Ductus Deferens

There is an epididymis and ductus deferens, but there is no head, body, or tail to the epididymis, which appears as a slight bulge on the testis. The tightly packed efferent ductules join to form the deferent duct, which arises from the caudal end of the epididymis; the ductus deferens closely follows the ureter to the middle chamber of the cloaca (urodeum). In sexually active cockerels the ductus deferens is full of spermatozoa and thus is whitish in colour. The copulatory apparatus (phallus) of the chicken consists of two papillae and a rudimentary copulatory organ that is located in the vent (Scanes et al. 2004).

Male Reproductive Physiology

Each testis is surrounded by a layer of connective tissue containing the seminiferous tubules and Leydig cells, which are dispersed in the spaces between the tubules. The interstitial cells produce several androgens, but the major hormone is testosterone. As sexual maturity is attained, the production of testosterone is stimulated by the rising blood concentrations of LH. In the sexually mature cockerel, the blood concentration of LH is maintained by a negative feedback loop in which the elevated concentrations of testosterone inhibit

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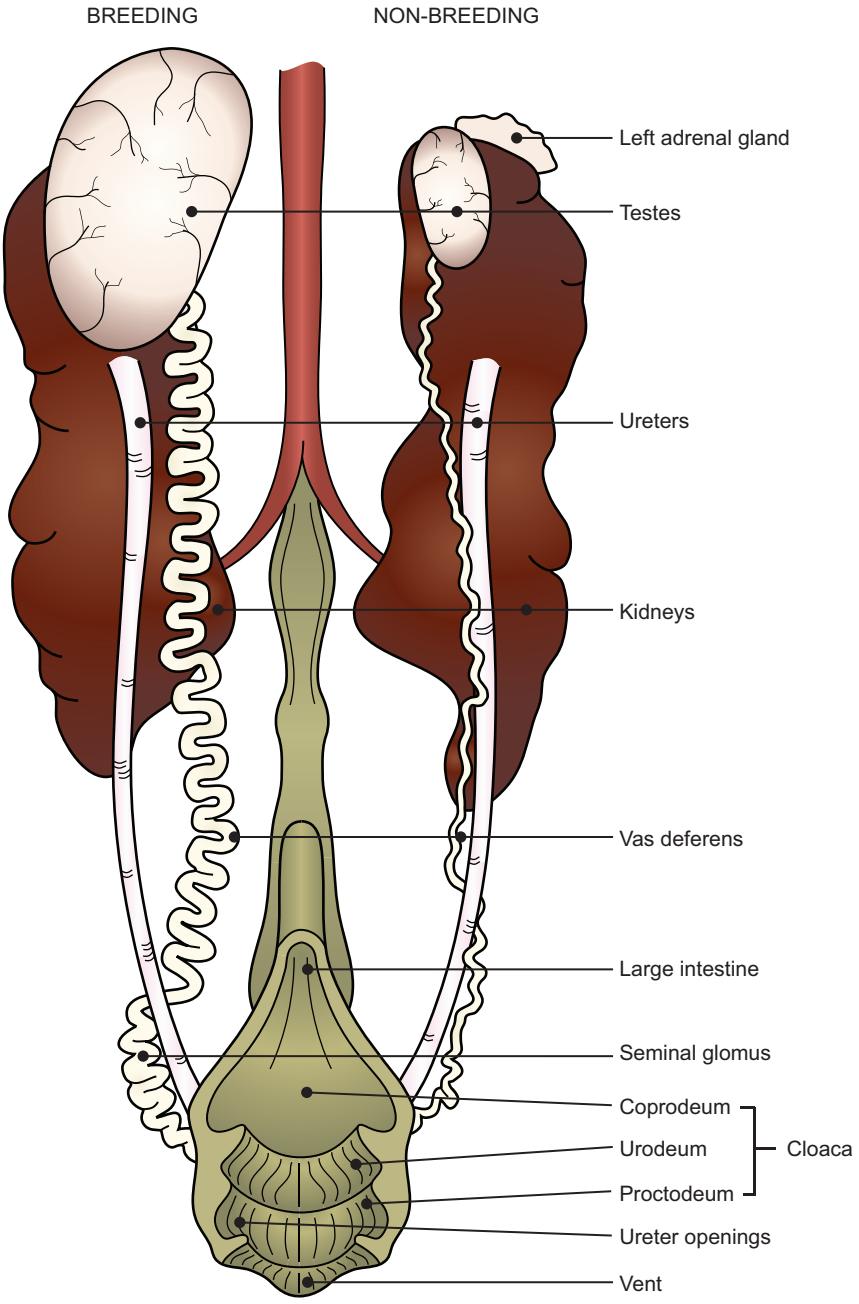

• **Fig. 30.7** A diagram of a cockerel's reproductive anatomy with the structures shown during breeding on the left and nonbreeding on the right.

the secretion of GnRH, which in turn inhibits the secretion of LH. As the secretion of LH declines, the concentration of androgens declines, and hence the secretion of GnRH and LH is enhanced.

The Sertoli cells provide the microenvironment in which differentiation can take place and act as nurse cells to the developing sperm; the duration of spermatogenesis is approximately 14 days.

Sperm Storage

There are sperm storage tubules at the junction of the vagina and shell gland (uterus). Sperm are ejaculated into the cloaca or vagina and rely on their motility to reach the sperm storage tubules (SSTs). Some then enter the uterus and are carried passively to the infundibulum where fertilisation occurs; sperm storage tubules can store sperm for a long time (10 days) because bird sperm remains viable at body temperature (41°C) for up to 2 weeks in the female's genital tract, resulting in successful fertilisation. For comparison mammalian sperm remains viable in the female reproductive tract for a few days at most and then only under ideal conditions. After an egg is laid, some of these sperm may get 'squeezed out' of the tubules into the lumen of the tract, from where they may migrate further up to fertilise another egg.

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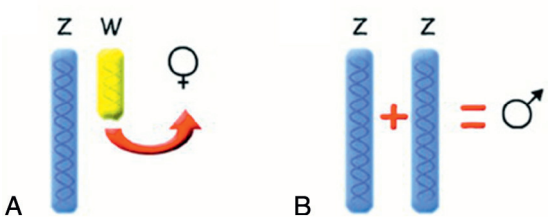


• **Fig. 30.8** (A) The heterogametic female avian chromosomes Z & W. (B) The homogametic male avian chromosomes Z and Z.

Fertilisation

Ovulation results in the release of an egg from a mature follicle on the surface of the ovary. At ovulation the ovum is captured by the infundibulum, which is covered with a ciliated simple columnar epithelium. The beating of the cilia is important in moving the egg into the funnel shaped/fluted upper end of the tube. After passive entry into the infundibulum by the egg, there is fertilisation when the sperm penetrates the ovum and there is fusion of the male and female pronuclei (syngamy). From here, the egg further develops during the 25-hour journey through the oviduct, with the end result being a fertile, shelled egg.

Sex Determination

Because all male birds are ZZ (homogametic unlike the heterogametic mammalian male XY), all spermatozoa will carry the Z chromosome (Fig. 30.8), whereas at the end of the second meiotic division, the Z and W chromosomes of the heterogametic avian female will have segregated into 50% of the ova. At fertilisation any of the ova carrying the Z chromosome will develop into male chicks, whereas any ova carrying the W chromosome with develop into the female chicks.

Egg Development

The infundibulum also has a secretory function. It produces the first of the egg coats, the chalazae. These are the whitish string-like structures on either side of the yolk that keep the embryo in proper position during development (University of Illinois Extension). Around the yolk, there are four distinct layers of albumen (Fig. 30.9):

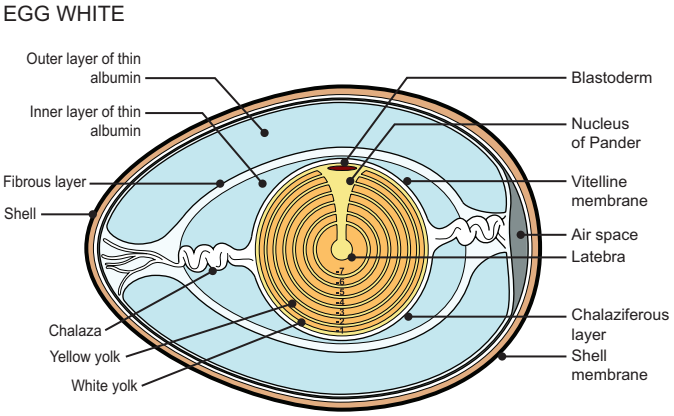
1. The chalaziferous (inner thick) layer attached to the yolk (3% by weight) – produced in the infundibulum
2. The inner thin (liquid) layer (~17% by weight) – produced in the infundibulum
3. The outer thick layer (~55% by weight) – produced in the magnum
4. The outer thin (fluid) layer (~25% by weight) – produced in the magnum

The inner and outer shell membranes are produced in the isthmus. There are three calcified layers to the eggshell.

The organic shell matrix is a series of layers of proteins and mucopolysaccharides in which calcification occurs. Just before oviposition (expulsion of the egg), the cuticle (a thin waxy layer) is applied to the shell.

Brooding/Broodiness

A backyard hen that lays 5 or 6 eggs, whether they have been fertilised or not, is likely to become broody if the clutch of eggs



• **Fig. 30.9** The anatomy of a poultry egg.

is not removed from her. The daily removal of laid eggs and the reproductive physiology of an indeterminate layer can result in over 300 eggs per hen being laid over a 12-month period. Physiologically, she is striving to establish a clutch of eggs but is being thwarted on a daily basis by the stock keeper. After a clutch of eggs are laid, a hen's behaviour changes, and she becomes broody. This may be a desired quality if the owner wishes a hen to incubate her own fertilised eggs; it may be an undesirable trait if the purpose of the hen is to lay eggs for human consumption or to produce fertile eggs for artificial incubation. To prevent broodiness, eggs should be collected daily, and there should be few dimly lit hiding places for a hen in her enclosure. From an endocrinological perspective the following is occurring:

- In the ruptured follicle, granulosa cells synthesize progesterone, and, if sufficient, it inhibits further ovulation.
- The ovary, oviduct, and comb regress.
- LH levels decrease during incubation and hatching but increase if the hen lays a second clutch of eggs.
- Gonadal steroids decrease, whereas prolactin increases.
- Prolactin increases in males and females during egg laying, peaks during incubation, and declines when chicks hatch.

The purpose of brooding is to keep the eggs warm. The hen develops a brood patch (Fig. 30.10) towards the end of the egg laying period, and the skin becomes oedematous and highly vascularised. Brood patches develop in response to ovarian steroid exposure. The tactile simulation of the defeathered, oedematous brood patch triggers transition from egg laying to brooding due to prolactin release. The blood flow to the brood patch is greatly increased, such that the heat from skin in this area incubates the eggs. Sensory fibres detect skin temperature where there is skin-to-egg contact. If the hen's body temperature falls she will shiver to generate heat and increase her metabolism to ensure that eggs are at the optimal temperature for incubation. The brooding period ends when prolactin levels decline.

Moulting

Moulting is a natural process in which the laying hen's reproductive function goes into abeyance and coincides with seasons of the year that are suboptimal for chick rearing. This is not a desirable trait for birds kept for egg production, but it may be of use to a small flock keeper who wishes to synchronise egg production. If that is the case, a flock moult can be induced by reducing the

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• **Fig. 30.10** The ventrum of a hen with a prominent red brood patch.

day length to 8 hours and restricting (but not withholding) feed. After a rest period, production can be resumed by then increasing the day length to 14 hours over 4 weeks before raising it to 16 weeks, and increasing the ration. If hens in a flock appear to be losing feathering commencing at the neck followed by breast, body, wings, and tail, in that order, in conjunction with a drop in flock egg production, then a moult is occurring.

Hatchery Management

Egg Storage

The aim of egg storage is to stop embryo development**,** and this requires a cool temperature: 17°C to 21°C (63°F–70°F), which should be ensured with careful monitoring using a minimum/maximum thermometer. However, there are still potential problems, such as hot spots in the egg store. Temperature fluctuation during storage can cause a decline in hatchability of up to 3.5%, and eggs stored for longer than 10 days have a decreasing hatchability of 1% per additional day. However, judicious use of an egg store allows pooling of eggs before setting.

Incubation/Setters

Fertilisation of the follicle is the first step in the production of a hatching egg.

The egg membranes and shell are then added over a period of approximately 24 hours, which is achieved *in vivo* at the hen's body temperature of 41°C. Therefore at the point of laying, the embryo is 24 hours old, and 1 day should be 'added' to the standard incubation periods for all species (i.e., 21 + 1 days for a hen egg). During this egg development period, the embryo progresses from one cell to 20,000 to 40,000 cells.

Incubation is divided into two processes: the 'setters' receive the eggs for the first 18 days, then for the final 3 days the eggs enter the 'hatchers'. For very small incubators (Fig. 30.11), eggs

• **Fig. 30.11** A small incubator.

are laid on their side, and the machine is a combined setter and hatcher. Such machines require a temperature of 38°C.

Single Stage Versus Multistage Incubators

A single stage incubator has all eggs at the same stage of incubation. The advantage of this design is that, potentially, the incubator can provide the optimum environment for development and hatching for each day of incubation. There is also improved biosecurity, as the incubator will be empty periodically for thorough cleaning and disinfection. A disadvantage is cost, as these incubators are often large, and they need either to supply heat to the eggs or to remove heat or cool them.

A multistage incubator has eggs at various stages of incubation. This reduces the cost of the operation, as some eggs need to be supplied with heat, whereas others need to dissipate heat. However, the machine is providing 'average' conditions for the age range of eggs. There is also a biosecurity compromise, as the capacity of the machine is being maintained at its maximum with more recently laid eggs.

Temperature Control

When monitoring a hatchery, the critical issue is embryo temperature; however the incubator air temperature may not necessarily reflect embryo temperature. It is known that egg shell temperatures correlate well with embryo temperatures if taken correctly with an infrared thermometer at the equator of the egg and therefore is better than a hatchery air temperature thermometer. The downside is that measuring the egg shell temperature may require opening of the machine, with a resultant dissipation of heat. From about halfway through incubation, an embryo changes from a net absorber of heat to a net producer of heat, though the optimal temperature for the embryo remains in the range 37.8°C to 38°C. This is a challenge for an operator of a small machine, as the focus changes from heat supply to heat dissipation. If the temperature is slightly suboptimal, then development is delayed, and if the temperature is slightly higher, target chick viability is compromised; if it goes even higher hatchability is also affected.

Humidity Control

The optimal operating humidity for a hatchery is 55% to 60%. When incubated correctly, eggs will lose 11% to 12% of their

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weight from the time of laying to day 18 of incubation. Small amounts of moisture loss occur during storage (approximately 0.5% per week), which need to be deducted from the incubation value. Egg weight monitoring is a valuable tool, but not commonly used (Aviagen Website). If the humidity is too high, then the egg's rate of moisture loss will be too low and the air cell too small; ultimately, the embryo cannot inflate its lungs. If the humidity is too low, then the rate of moisture loss will be too high, and thus there is a risk of embryo dehydration.

Movement Control

Egg turning is paramount for proper embryo development, as it prevents the embryo adhering to shell membranes. There is also better yolk sac and allantoic vascular development, better albumen protein utilisation, better hatch weight, and better embryo positioning before hatching. Turning in the first week is more important than in the second week and should be done at least 5 to 6 times per day. The eggs should not be turned in the last 3 days of incubation after transfer from the setter to the hatcher.

Ventilation Control

Air exchange is essential to prevent suffocation of the embryo. Removal of carbon dioxide (CO_2) is required as a concentration of 1% CO_2 has a marked detrimental effect on hatchability. However, in normal avian nests, CO_2 is 10 times higher than atmospheric CO_2 levels (0.4%–0.6% vs 0.04%). The operator's aim should be to ensure as much ventilation as possible can be provided while maintaining adequate temperatures. Ventilation is a major method of temperature control for the hatching egg; if it is too hot, this increases the rate of embryonic development, potentially affecting chick quality. If it is excessively too hot, there will be embryo mortality, resulting in reduced hatchability. Too cool a hatchery delays the rate of embryonic development.

Temperature fluctuations cause embryo 'stress' – reduced hatchability, reduced chick viability. Hot and cool spots (e.g., due to egg size variability, air movement issues) will result in an increased hatch window (the duration of the interval from the first to the last egg to hatch), which influences chick viability (Engormix Website).

Microbial Control

If eggs have a low total viable count of bacteria before incubation, then contamination should decline during the process (Fig. 30.12). Highly contaminated eggs placed in dirty machines will result in bacterial proliferation, with detrimental consequences on hatchability and chick viability.

Candling

A bright light can be shone through the egg (Fig. 30.13) enabling the viewer not only to see if the egg is fertile and developing but also whether it has the correct amount of humidity. This is done by assessing the relative size of the air cell.

	Pre-incubation	At transfer to hatcher
Clean Egg	5×10^2 - 1×10^4	$< 1 \times 10^2$
Dirty Egg	2×10^4 - 2×10^6	2×10^7

• **Fig. 30.12** Low egg bacterial contamination will get lower during incubation whereas high levels of egg bacterial contamination will get higher during incubation.

Incubation/Hatcher Settings

Candling is often carried out before transferring eggs from the incubator to the hatcher at day 18 of incubation. This should be done with great care to avoid damage to the developing fetus. Once in the hatcher, the eggs no longer need turning. The temperature requirement is now lower for the egg, but the humidity requirement is higher. With gas production and bacterial contamination, there is always a risk of what are described as 'bangers', namely exploding contaminated eggs with the consequential risk they pose to other eggs in the hatcher. With each batch of eggs being hatched, there should be an aim to have a short period of time when there are no eggs in the hatcher; this is referred to as a 'hatch window'. The longer the hatch window, the greater the welfare compromise and the disease risk to the first hatched chicks. A hatch window of 24 hours should be achievable. Excessive amounts of meconium present in a hatch basket would alert a veterinary surgeon to there being a long hatch window.

Sexing

DNA Sexing

It is possible to harvest tissue from the young chicken for karyotyping. There are several laboratories in the UK that offer this testing service. Sampling techniques include:

1. Blood collection by venipuncture or clipping a toe nail so that a few drops of blood can be placed on a permacode card (Fig. 30.14); the card should be left to dry for approximately 20 minutes at room temperature. Once the card is dry, it should be placed in a small envelope with the submission form and sent to the appropriate laboratory.
2. Two or three feathers can be plucked from the bird (they cannot be naturally shed feathers, as they have only keratinised material with no DNA). Although any feather can be selected, as a general rule, they should be between 5 to 10 cm (2–4 inches) long. They are then placed into a sealed plastic bag to be sent to the laboratory.
3. After hatching, the shell remnant can be used for sexing the hatched chick. Allow the eggshell membrane to completely dry before submission. Shells may be left in the incubator or removed and allowed to air dry for 24 hours or until the egg membrane is no longer moist; removing the moisture will

• **Fig. 30.13** Candling of fertile egg. (Courtesy of Boehringer Ingelheim.)

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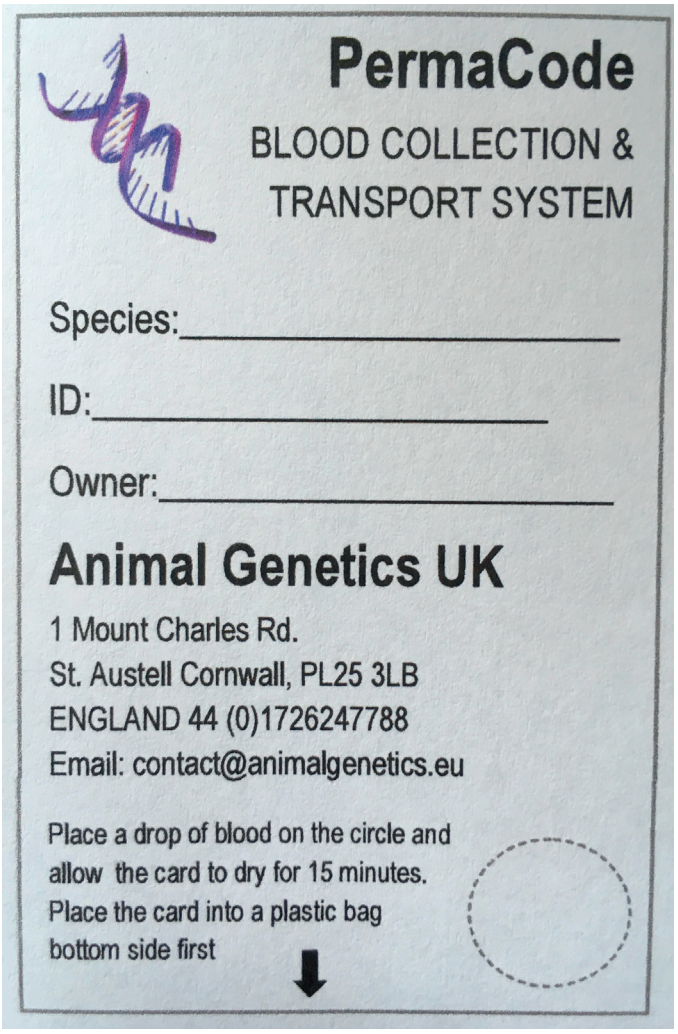

• **Fig. 30.14** A permacard for submitting a blood sample for DNA sex determination testing.

slow down the decay of the DNA required for testing. DNA is extracted from the vascular material lining the eggshell membrane. This testing method is very accurate.

Surgical Sexing

It is possible to surgically sex birds, although not in very young birds, and it is not without risk. The patient requires general anaesthesia before the insertion of an endoscope through a surgical incision between the ribs at the level of the caudal thoracic air sac.

Faecal Hormone Measurement

Fresh faecal samples can be assayed for testosterone and oestrogen concentrations in sexually mature birds. The chickens being tested must be healthy and the sampled faeces assigned to the correct individual; the test is safe and inexpensive.

Vent Sexing

Due to the slight anatomical difference in the male and female vent, it is possible to determine the sex of day old chicks with this technique. However, it needs much training to become proficient, as the anatomical differences in male and female vents are far subtler than other species such as ducks in which even at a day old the male phallus is quite obvious.

• **Fig. 30.15** Autosexing: comparing flight feathers of female (on the left) to male (on the right) chicks. (From Aviagen Ltd.)

Autosexing

In some breeds of chickens, there are some easily observable sex-linked characteristics allowing identification of the sex of the chick on hatching.

1. Colour sexing. For example, the Barred Plymouth Rock breed carries a gene for barring, which produces a white bar on a black feather. Males have a large white head spot that is smaller and narrower in females. Furthermore, if a gold coloured male is mated to a silver female, the progeny will consist of silver males and gold females.
2. Feather sexing (Fig. 30.15). This is possible due to rapid or slow feathering and the subsequent differences in wing feathering. If rapid feathering males are mated with slow feathering females, the result is slow feathering males and rapid feathering females (i.e., males have shorter wing feathers).

Failure to Lay/Egg Drop

There are numerous causes of reduced egg production, including infectious, nutritional, and environmental factors.

Egg Binding and Dystocia

Egg binding is an unhelpful term, as many practitioners and producers have different views on its precise definition. Hens can suffer from dystocia in which an egg becomes trapped in the vagina. The trapped egg may be visible on examination and will certainly be palpable. Large eggs (especially those with two yolks) are a contributing factor, as are hypocalcaemia, obesity, and early onset of lay. It may be possible to assist the expulsion of the egg with adequate lubrication. Initially, intramuscular calcium supplementation can help in the form of calcium gluconate followed by calcium glubionate or calcium carbonate orally. Some veterinary surgeons advocate breaking the offending egg. However, though this may rectify the immediate problem, it does predispose the bird to developing salpingitis. If the flock is laying a high percentage of double yolked eggs,

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a review of the lighting schedule is required. A nutritional review should also be considered to ensure the hens are not overweight and the egg weight is not higher than breed recommendations.

Salpingitis

Inflammation of the oviduct is often encountered due to ascending infections from the vent and cloaca. The incidence of this syndrome is increased in older hens, producing large eggs after a sustained period of production. Most commonly, *E. coli* are isolated from an infected oviduct. However, these may be opportunistic pathogens with a harder-to-isolate bacterium or virus being the primary pathogen.

Oviductal Impaction

An end point to dystocia or salpingitis can be oviductal impaction in which egg material (such as yolk, albumen, mucin) and purulent material occlude the oviduct and can ultimately lead to pressure necrosis of the oviduct wall. Birds that have oviductal impaction have been in lay before ceasing production and becoming progressively lethargic, depressed, and anorexic. It tends to be seen in older hens and may be regarded as a normal end point to production in the spent hen. Palpation or imaging of the caudal coelom can detect the caseous mass in the oviduct. Antibiotic treatment tends to be unrewarding and ovariohysterectomy a touch heroic.

Oviductal Prolapse

Excessive positive pressure in the coelom, such as a discrete mass or excessive straining, can lead to an oviductal prolapse. If it does so, the individual is at risk of cannibalisation by other hens in the flock. Former commercial laying stock tends to have been beak trimmed, which can limit the severity of the cannibalism, but this procedure is rarely performed on pedigree/pet poultry. Even if this does not occur, the condition is a surgical emergency; however, euthanasia should be considered on welfare grounds. If this condition is prevalent in a flock, consideration should be made to providing more available nest boxes.

Uterine Torsion

Uterine torsion occasionally occurs and normally proves fatal for the bird. There is often vascular compromise, and the oviduct will be oedematous, reddish black in colour, and friable.

Cystic Hyperplasia of the Oviduct

Numerous cystic structures can occur; they may contain clear or cloudy fluid and are lined by proliferative mucosa of the particular portion of the oviduct involved. This is probably an endocrinopathy, but the exact cause is not known.

Cystic Right Oviduct

It is not uncommon to see oviductal cysts of the vestigial right oviduct, although two Müllerian ducts are present in the developing chick embryo. The left duct develops normally into the oviduct, and the right duct regresses; occasionally regression does not occur, and it becomes a dilated fluid filled cyst. It can be especially pronounced in the sexually immature pullet, although it is an incidental finding as they do not appear to inhibit subsequent sexual development or cause disease.

Blind Layer Syndrome

Infectious bronchitis (QX strain) is known to cause blind layer syndrome (Pattison et al. 2008). For this to develop, the birds need to be infected when young chicks, before development of the oviduct. There may be partial, or almost complete, absence of the duct or vestiges that are nonpatent or cystic. Once sexually mature, the hen will ovulate, but the ovum is unable to pass normally through the oviduct and can be shed into the body cavity. The hen goes through the process of oviposition but fails to lay (hence blind layer syndrome). Affected hens have pendulous, fluid-filled body cavities and an obvious penguin-like gait. On postmortem examination, the ovary is normal but the affected oviduct is thin walled and cystic and can contain up to 1.5 litres of fluid.

Ectopic Ovulation

Ectopic eggs can be an incidental finding but can also be a cause of mortality, particularly if associated with peritonitis. Causes of this condition include oviductal rupture and reverse oviductal peristalsis.

Egg Peritonitis/Reproductive Associated Coelomitis

If the oocyte is not harvested by the infundibulum at ovulation, it may float freely in the body cavity. Rough handling of a hen may also dislodge other follicles from the ovary. It is possible that there is some reabsorption and no infection because free yolk is very irritating to the peritoneal lining of the coelom. This, or a progressively more severe salpingitis, can develop if egg material from the reproductive tract enters the coelom and becomes infected, leading to an egg peritonitis (Fig. 30.16). Affected hens are depressed and inappetant. If this condition becomes chronic, there may be involution of the ovary. Radiographs may show multiple radiopaque densities in the peritoneal cavity, though diagnosis is more frequently confirmed on postmortem examination. Coelomocentesis will yield fluid (unless the lesion is very consolidated) containing yolk material, inflammatory cells, and bacteria. If a flock is very hyperactive, husbandry measures should be taken to calm them; otherwise, egg peritonitis will occur at an unacceptably high level.

• **Fig. 30.16** Egg peritonitis with fibrin tags associated with a mature ovary.

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Vent Gleet

Infection of the vent is called 'vent gleet'. A vicious cycle can be established in which there is initial trauma to the vent with subsequent infection, resulting in cannibalistic behaviour and further trauma; it can occur in both hens and cockerels.

Oophoritis

Inflammation of the ovary can be secondary to infection of the peritoneal cavity or air sacs or associated with septicaemia. Viruses, bacteria, fungi, and mycobacteria are all possible causes. Ovarian lesions with intense blood vessel congestion and deformed shrunken ovules have also been associated with *Salmonella enteritidis* infection in laying hens (Pattison et al. 2008). If a salmonella species is isolated from infected birds, in the UK this must be reported to the Animal and Plant Health Agency (APHA), as part of the national control plan for salmonella; treatment with antibiotics is not permitted.

Ovarian and Oviductal Neoplasia

A drop or cessation in egg production could be attributed to ovarian neoplasia. Several different types of neoplasia have been reported, but they are rare. Some neoplasia can have a viral aetiology – for example Marek's disease. Confirmation of the diagnosis is made on histopathological examination of the affected ovary.

Egg Drop Associated With Lighting Pattern

Increasing day length stimulates reproductive development and production, whereas decreasing day length has the opposite effect; thus artificial lighting regimens are used to mimic these changes and stimulate production. This can be difficult in small flocks in which the housing provided often allows for light pollution, and the producer can be unsure of the optimal light duration and intensity.

Caged Layer Fatigue/Calcium Tetany

Affected hens show signs of paresis, and, although the hens may be in lay, they will have soft long bones. The flock will have a reduction in egg yields and poor shell quality. Supplementation with oyster shell can be beneficial.

Egg Eating

If the husbandry of a small breeding flock is suboptimal, it is possible for some of the hens to develop the vice of egg eating. It can be difficult to identify the culprits and to ascertain the action needed to stop the vice.

Fertility Issues

Keepers of small breeding flocks should be aiming for fertility levels of over 90% and hatchability levels of over 75%. If there is a failure to hatch, the first question is whether the egg is fertile. Breakout work on unhatched eggs (Fig. 30.17) can be useful as it is possible to ascertain at which point in time embryonic development ceased. However, the aviculturist is often unaware of embryonic death; thus the egg is left in the incubator beyond the time of death, leading to severe autolysis.

An infertile yolk is virtually homogenous in appearance apart from a small white area that is the blastodisc. The presence of large numbers of them should cast suspicion on the cockerel's fertility. Infertility can be due to lack, or poor numbers, of viable sperm or the inability to mate properly. In small flocks it is possible to identify hens that have been repeatedly mated; they will often have feather loss over their dorsum and neck, as well as damage to the integument. Conversely, it is also possible to identify those that have not been mated. A pullet that has not laid an egg will have a narrow gap between the points of the ischium. If a handler can position the width of two fingers or more between these points, the hen will have laid an egg.

After the monitoring of embryonic death, a late peak (18–21 days) close to hatching will be identified. Normally, there is also an early peak in embryo mortality (0–4 days) when there is the period of cell differentiation and this is commonly associated with egg handling (possibly before placement in the incubator). Excessively high incubation temperatures and excessive movement of the eggs can also be implicated in these deaths. Midterm embryonic death is relatively uncommon.

Late embryonic death is likely to be associated with the physiological complication changes of switching to pulmonary respiration. For an egg to hatch, it is critical that it loses a certain amount of moisture, which, if not achieved, will result in embryonic death. Very late embryo mortality is a hatching issue and is manifested as pipped shells in which the chick has attempted hatching but failed. These eggs may have been incubated beyond the range of acceptable temperatures.

A few days before hatching, the shell membrane contracts around the chick making the air cell larger, and 24 to 48 hours before hatching, the chick positions itself with its head in the air cell, allowing it to breathe. Abnormal positioning is potentially life threatening and can result from improper turning of the egg during incubation.

Orchitis

Inflammation of the testes is rare. However, if there is an infertility issue in a flock, it should be considered as a differential diagnosis. On postmortem examination of an affected cockerel, the testis/testes may be enlarged, reddened, and with multifocal lesions. Infectious causes of orchitis include fungal, bacterial (such as mycobacterium and chlamydia), and viral infections (e.g., infectious bronchitis).

Testicular Neoplasia

Though various types of testicular neoplasia have been reported in birds, they are rare in backyard poultry (Schmidt et al. 2015). In a small flock with one cockerel, it could explain a drop in fertility. Diagnosis would be on gross postmortem examination, when enlarged testes would be seen, followed by confirmation on histopathology.

Poor Libido

Immature cock birds, reared separately from females, will subsequently have a low libido when introduced to a breeding flock. Submissive cockerels in a flock can be bullied by both dominant males and females, thus influencing their breeding capability. Not only are male to female ratios important in a breeding flock (~1:8) but also the establishment of a nonviolent pecking order.

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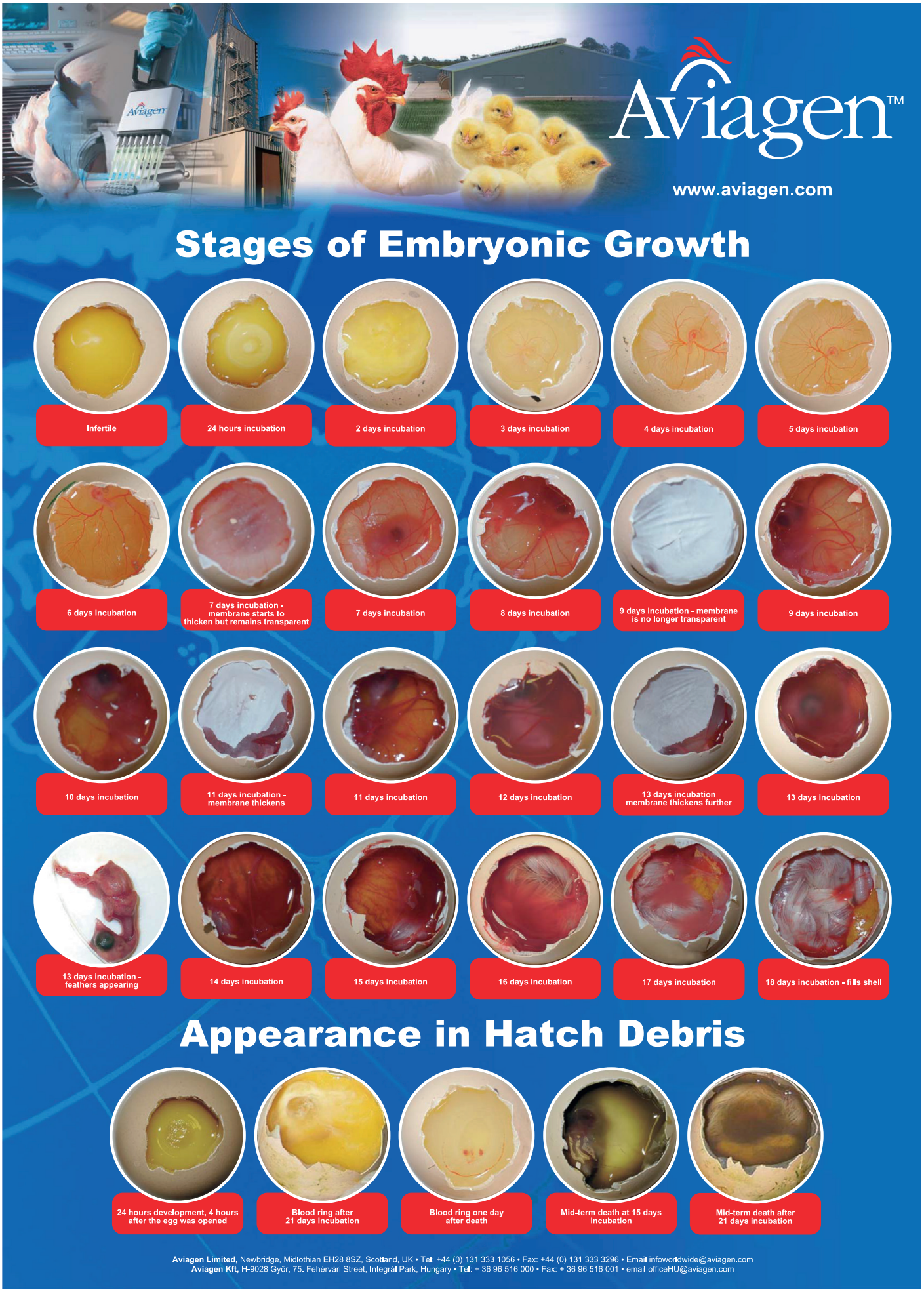

• **Fig. 30.17** Break out work on fertilised eggs showing fetal development during incubations. (From Aviagen Ltd.)

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• **Fig. 30.18** An ex-commercial layer showing upper respiratory tract clinical signs due to infectious bronchitis.

Infectious Causes of Reproductive Disorders

Infectious Bronchitis

A drop in egg production in a nonprotected flock caused by infectious bronchitis (IB) can be up to 50%. The pathogenicity is dependent on the strain of coronavirus causing the infection. Production can return 4 to 6 weeks after the initial challenge, though this often coincides with a rising number of pale, crinkled, weak shelled eggs; internally, the albumen will be watery. There can be concurrent respiratory signs (Fig. 30.18) and mortality. In immature flocks an early onset infection with IB QX strain is the cause of blind layer syndrome. On postmortem examination, birds from an infected flock may have lesions typical of egg peritonitis.

In situations in which it is not essential to isolate the virus, PCR is the test of choice for confirmation of diagnosis. Serological testing using either haemaglutination inhibition (HI) or ELISA can also be used. If a strain of IB is identified as the cause of a drop in egg production, there is no specific treatment. However, the flock keeper and veterinary surgeon should consider control by vaccination. Although commercial vaccines have 1000 doses as the smallest vial size, the live vaccines are relatively cheap and easy to use in small flocks.

Mycoplasma Gallisepticum

Mycoplasma gallisepticum (MG) can be spread both horizontally and vertically. These bacteria survive well in the allantoic fluid and yolk. A laying flock infected with MG will show signs of upper respiratory tract disease (especially severe conjunctivitis and sinusitis) with a concurrent drop in egg production. Infected layers will have a caseous exudate in the oviduct. There are no pathognomic lesions, but there are three laboratory test approaches to diagnosis: antibody detection, isolation of MG, and detection of its DNA. With high levels of biosecurity and good screening programmes, eradication of MG has been possible in commercial primary breeding flocks. With a severely affected backyard flock, culling should be considered. If this is not viable, some level of control is possible with metaphylactic antibiosis and vaccination; both live and inactivated vaccines are available.

Mycoplasma Synoviae

Like MG, mycoplasma synoviae (MS) has a short survival time outside the host and similar modes of transmission; some flocks infected with MS remain asymptomatic. Others can show signs of lameness, mild lower respiratory signs, and a drop in egg production with some eggs showing signs of egg apex abnormality. Diagnosis is by similar methods to MG; live vaccines are available for control.

Egg Drop Syndrome

Although commercial hens are vaccinated against the adenovirus infection, egg drop syndrome (EDS-76), most small backyard flocks are not. Eight days postinfection, there is a massive growth in the pouch cell gland region of the oviduct, coincident with the occurrence of egg shell changes. Progeny from these infected eggs can hatch and appear clinically normal but latently infected. When a latently infected individual reaches peak lay, the virus is reactivated. The virus can be transmitted vertically and should an unprotected flock become infected during lay, the egg drop can be profound. Transmission is possible horizontally via the faecal/oral route. Classically an infected flock will initially show a loss of shell pigmentation, followed by thin shelled, soft shelled, and shell-less eggs. There is an apparent drop in egg production, though overall production may be normal, but there will be fewer normal eggs. It has been calculated that the loss equates to about 10 to 16 eggs per hen when a naïve flock is infected (Pattison et al. 2008). Confirmation of a challenge is possible with rising antibody titres on paired serology or PCR testing (assuming isolation of the virus is not required). No treatment is available, but even in small flocks, vaccination should be considered. The only licenced EDS vaccines in the UK and internationally are polyvalent inactivated vaccines. Replacement pullets for an infected site should be injected with this vaccine intramuscularly between 14 and 16 weeks of age.

Notifiable Diseases in the UK and Internationally

Both avian influenza and Newcastle disease are notifiable diseases of poultry, and both can produce a drop in egg production as a clinical sign. With highly pathogenical avian influenza (HPAI), there can be complete cessation of lay in a flock in conjunction with high levels of mortality and morbidity. It must be remembered that low pathogenical avian influenza (LPAI) (H5 or H7) is also notifiable. Clinical signs can be far subtler with much lower levels of mortality and drop in egg production rather than a cessation. Paradoxically this makes LPAI more difficult to diagnose than HPAI and a differential diagnosis for any flock with a drop in egg production. There are no pathognomic lesions for avian influenza on gross postmortem examination, with the carcass merely having lesions suggestive of an acute or peracute viraemia.

Clinical signs for a flock infected with Newcastle disease virus (NDV) are dependent on the pathotype of the NDV. The appearance of shell-less or soft shelled eggs, often laid outside the nest boxes, followed by complete cessation of lay, is a common presentation. With highly virulent forms, high levels of mortality will occur. There can be intestinal, neurological, and respiratory signs, depending on the strain. Like avian influenza, there are no pathognomic lesions. However, haemorrhagic lesions would arouse suspicion especially if the mucosal surface of the proventriculus was affected.

Early detection by veterinary surgeons of these diseases is important for national disease control but can be very difficult for the nonpoultry expert dealing with a small flock. If a suspicion of these diseases persists after an initial investigation, the veterinary surgeon must contact APHA. In cases of avian influenza in which the clinical signs are not profound and after discussions with APHA, the private veterinary surgeon may elect to test in order to exclude

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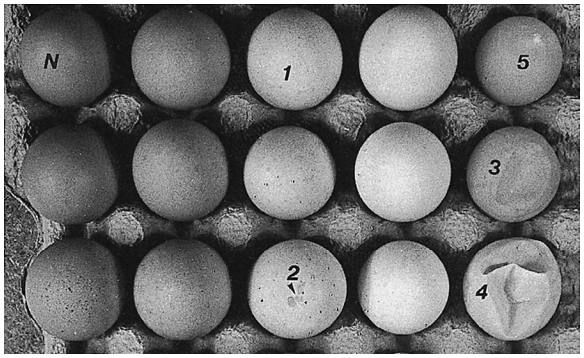

• **Fig. 30.19** A tray of downgraded eggs infected with EDS. These changes range from the normal brown eggs (N), to the loss of shell pigment (1), thinning of the normal eggshell (2), cracks where the eggs are damaged in the nest box or handling to soft-shelled (4), and shell-less (5) eggs. (From Pattison M, *Poultry diseases*, 6th Ed, Philadelphia, 2008, Saunders.)

• **Fig. 30.20** *Ascaridia galli* having migrated to the yolk of a subsequently shelled egg.

avian influenza, rather than officially notify (Testing for Exclusion of Avian Notifiable Diseases).

Abnormal Eggs/Seconds

Abnormal egg shells are often referred to as 'seconds' as they are downgrades from first class eggs. Numerous abnormalities are recognised (Fig. 30.19), some of which have known aetiologies, whereas others are suggestive of particular conditions. Though egg shell abnormalities are often a result of nutritional or infectious disease in the hen, it should be borne in mind that poor egg handling and storage can lead to damage. Whatever the cause of the egg shell abnormality, invariably there will be a detrimental effect on hatchability.

Double Yolked Eggs

Though this is can be regarded as a bonus in eggs for human consumption, in breeding programmes, they are an important issue, as no double yolked egg has been known to be fertile. Prevalence is higher in young flocks in which up to 20% of eggs can be affected. The condition is exacerbated by prolonged day length and light intensity; excessive intake of methionine can also induce this condition. This phenomenon does put the young hen under additional physical strain, as double yolked eggs are larger than single yolked eggs.

Cracked Eggs

The causes are rough handling by the birds, the equipment, or the flock keeper; it is exacerbated in poorly mineralised, large eggs.

Soft Shelled Eggs

Some viruses can cause soft shelled eggs, notably adenovirus and infectious bronchitis virus. They can also be a manifestation of vitamin A, vitamin D or calcium deficiency.

Shell-Less Eggs

In nest boxes, fully formed eggs minus the shell can be found. Infectious diseases known to damage the oviduct may be implicated, which include infectious bronchitis, egg drop syndrome, and *Mycoplasma gallisepticum.* Nutrient imbalances in calcium, phosphorus, and vitamin D3 may also be responsible.

Discoloured Yolks

Nutrition plays a major part in yolk colour. Feed additives may enhance the richness of the yolk colour (e.g., carotene rich rations) or diminish the yolk colour (e.g., oxidisation of carotenoid feed additives). If blood spots are detected in yolks, it is assumed that the bird has been exposed to some acute stress, such as a thunderstorm or predation. Vitamin K deficiency may also be a factor.

Meat spots in the yolk are due to pieces of ovarian or oviduct tissue becoming embedded in the yolk. There appears to be a genetic component to this condition, as it is more prevalent in brown than white hens.

Albumen Quality

Watery albumen, revealed on cracking an egg, is often indicative of an active viral infection.

Crinkled Eggs

This condition is often associated with an infectious bronchitis challenge.

Vitamin Imbalance Effects on Shell Quality

Inadequate vitamin A and D levels in feed may lead to thin egg shells. A sandpaper texture to eggs may be associated with inadequate levels of vitamin A and/or vitamin D.

Worms in Eggs

Very occasionally, the intestinal nematode *Ascaridia galli* can migrate from the cloaca up the oviduct to become encapsulated in a shelled egg (Fig. 30.20).

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Pale Eggs

White hens tend to lay white eggs, and brown hens tend to lay brown eggs. The protoporhyrin-IX pigment that is secreted from the epithelial cells lining the uterus during the 90 minutes just before oviposition is responsible for the brown eggshell colour. Loss of pigment resulting in pale eggs can be seen with certain stressors, such as an infectious bronchitis challenge and the sudden exposure to high levels of UV light.

Small Eggs

Flocks that are 'lit up' with a lighting programme that brings them into lay too early will be afflicted with the production of small eggs.

Blood Stained Eggs

If blood stained eggs are noted, the veterinary surgeon has to ascertain whether the blood is from the distal intestinal, the reproductive tract, or the cloaca/vent.

Egg Apex Abnormality

As previously mentioned, egg apex abnormality (EAA) is highly suggestive of a *Mycoplasma synoviae* infection.

Late Fetal and Chick Pathology

Late fetal deaths and neonatal deaths can be caused by a variety of nutritional, genetic, and infectious issues. Postmortem examination of such cases can reveal oedematous carcasses and is often a result of excessive hatchery humidity. Should the humidity be too low, then the carcasses are seen to be very dehydrated. Bacterial infections are often associated with poor husbandry and cleanliness, which can be traced back to the breeder flock, egg store, hatchery, or brooding hut. Pathogenical *E. coli* infections are the most common cause of lesions to the umbilicus and yolk sac. The umbilicus can be large, swollen, and reddened with the body wall becoming oedematous and discoloured; if this is extensive, the specimens are referred to as mushy chicks. The yolk sac can also be involved, being enlarged, discoloured, and oedematous; this is distinct from retained yolk sacs. In these cases, the chicks will not thrive, but the large yolk sac is a normal colour and not contaminated.

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Equine Infertility and Stud Medicine Practice

DALE PACCAMONTI AND JAMES R. CRABTREE

The objective of the veterinarian working in any horse breeding enterprise, regardless of size, should be to produce the maximum number of live, healthy foals from the mares bred during the previous season; we could add to this 'as early as possible' in many breeding programmes. Perhaps the biggest obstacle to achieving this aim is the infertile or problem breeding mare. Very few mares are permanently and completely infertile, but subfertility of varying degrees is a major problem. There are many causes of subfertility that warrant a mare to be categorised as a 'problem breeding mare'. and although it is important to recognise the underlying cause, the implementation of a successful treatment strategy is equally important. Mare owners frequently have problem mares from which they want to produce foals, and the veterinarian must be able to help maximise the chance of this wherever possible. It might take several oestrous cycles to establish a pregnancy, and even then, there is an increased possibility of pregnancy failure. Commitment from both mare owner and veterinarian is needed; the owner should be made aware of this at the outset and be given a realistic expectation as to the chance of success. The aim of this chapter is not only to describe causes of infertility but also to consider the subfertile 'problem mare' and how to provide an effective management policy that can be applied in daily clinical practice.

There are two major ways in which human intervention has negatively influenced breeding success:

1. Reproductive performance is usually not the prime criterion in breeding horses.
2. For many breeds, e.g., Thoroughbred and Quarter Horse, 1 January is the official birthdate for foals in the Northern hemisphere (1 August in the Southern hemisphere) irrespective of their actual date of birth within that year. Because the natural breeding season in the horse centres on the summer solstice (21 June), horse breeders are plagued with problems in attempting to breed mares in the winter and early spring outside their most fertile period. The promotion of yearlings in the autumn sales contributes to this pressure for early breeding because well-grown, older yearlings tend to sell for higher prices. In many breeds, competitive events between 2 year olds are commonplace, and a horse that is 3 or 4 months older than others in the event is at an advantage.

Infertility in the mare can be a herd problem or an individual mare problem and can be very frustrating. From the viewpoint of herd fertility, it is important to review records to understand the degree of infertility or subfertility that actually exists, as opposed to the client's perception. Some grasp of normal expectations of fertility is useful. Assessment of reproductive efficiency is an extensive subject, and for a more detailed review, the reader should refer elsewhere (Hearn 2000, Love 2003, Allen et al. 2007). Possibly the two most important, or at least the two most quoted, parameters in connection with reproductive efficiency are: end-of-season pregnancy rate and live foal rate. From an economic perspective, these may be the most important benchmarks; however from the veterinarian's perspective, pregnancy rate per (oestrous) cycle is a more up-to-date indicator of the efficiency of the breeding operation. A well-managed stud farm should typically achieve pregnancy rates (diagnosed at 15 days of gestation) per cycle of 65%, an end-of-season pregnancy rate of 85%, and a live foal rate in excess of 75%. These figures may be lower than owner's or stud manager's expectations; therefore it is a good idea to discuss what they are at the beginning of the breeding season. The figures may need to be reviewed in the light of the history and past breeding performance of a particular mare (see Box 31.1). Pregnancy rates at the end of the season will depend on the fertility of the stallion, the fertility of mares, and management. This last factor is often related to the value of the horses involved because frequent veterinary attention, in cases in which it is justified by the potential value of the mare and future foal, results in better outcomes. Very expensive stallions tend to attract more fertile mares, or the stud may accept only young fertile mares. Well-managed studs tease mares regularly and individually; this is very time consuming yet hugely advantageous. An experienced stud manager knows, for example, the reasons why some mares fail to exhibit oestrous behaviour. In turn, the length of time that a mare fails to show oestrus before being presented to the veterinarian for examination depends on stud policy and the owner's wishes.

The clinician should be aware of how to investigate the problem breeding mare. A protocol for such an investigation of an infertile or subfertile mare is outlined in Box 31.2.

Causes of Infertility

Failure to Cycle Normally

A frequent history of a mare presented for infertility is that she either does not cycle or does not cycle normally. The first condition that must be ruled out in the former is pregnancy. There are a number

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• BOX 31.1 Factors to Include When Obtaining a History During an Infertility Investigation of a Mare

Age
Previous use
Current use
Intended use
Any pertinent health history
Has she ever received any hormones (or vaccines) for training or behavioural modification?
Has she cycled normally in the past?
Has she been bred (natural cover, artificial insemination; fresh, cooled, or frozen semen; fertile stallion)?
Has she ever been pregnant? Outcome?
Has she ever foaled (any problems during delivery, any postpartum complications)?

• BOX 31.2 Outline of Protocol for Clinical Examination of an Infertile Mare

Routine Procedures in All Cases:

1. Obtain previous breeding history.
2. Assess physical condition, general health.
3. Evaluate perineal conformation.
4. Examine reproductive tract by palpation per rectum.
5. Examine reproductive tract by transrectal ultrasound.
6. Obtain culture of clitoral fossa, clitoral sinuses.
7. Examine vagina and cervix visually with speculum.
8. Perform manual examination of vagina and cervix (while performing next step).
9. Obtain culture and cytology of uterine lumen (possibly using low volume lavage technique).
10. Obtain endometrial biopsy.

Procedures Performed in Selected Cases:

1. Perform hysteroscopic examination of uterine lumen.
2. Collect peripheral venous blood sample for hormone analysis.
3. Collect samples (e.g., blood, hair follicle, tissue biopsy) for chromosome analysis.

of situations, such as in a recently purchased mare, or when a mare or a stallion escaped from their living area, or when a mare was housed with a colt that was considered too young to be able to breed, in which mares may be pregnant without the knowledge of their owner. Although normally a pregnant mare does not exhibit oestrous behaviour, a small percentage of mares may exhibit signs of oestrus when pregnant. Obviously, pregnancy must be ruled out before any invasive diagnostic procedures are performed.

Seasonality

Another common cause of mares failing to exhibit regular oestrus is due to seasonal cyclicity. Although mares near the equator do not show a clear seasonal cyclicity, those at higher latitudes typically enter into a period of anoestrus during the winter when the photoperiod is short. Ovaries are small and inactive and the uterus and cervix are typically flaccid. Follicles, if present, are typically less than 15 mm in diameter, and serum progesterone is less than 1 ng/mL. Some, usually older, multiparous mares, may exhibit signs of behavioural oestrus when teased with a stallion. However, examination will reveal reproductive quiescence. The arbitrary birthday of 1 January for horses in the Northern hemisphere and 1 August for those in the Southern hemisphere pressurises breeders and veterinarians to ensure that mares become pregnant in the winter and thus have foals born as close to their arbitrarily imposed 'birthday' as possible. The problem is that mares, being seasonal long-day breeders, would normally be in anoestrus at that time of year. In fact, the average date of the first ovulation of the year for mares in the Northern hemisphere is 7 April (Sharp 1980). Therefore much effort has been made to induce cyclicity earlier than would normally occur. Stud farm managers who do not use such schemes find themselves experiencing subfertility due to a reduced number of breeding opportunities before it becomes 'too late' to breed from the perspective of the mare owners.

This endogenous rhythm cannot be changed in the short term, but with time, it can be modified. By exposing the mare to an artificial long daylight before the winter solstice, cyclic activity can be induced at an earlier date. Exposing mares to a minimum of 10 lux of light for a minimum of 14.5 hours (9.5 hours of darkness) starting from 1 December will stimulate mares to cycle (have their first ovulation) in approximately 70 days. In practice the authors recommend 16 hours daylight and 8 hours darkness. The light should be added at the end of the day rather than in the morning to be effective. Alternatively a 1 hour pulse of light during the night, 9.5 to 10.5 hours after the onset of darkness (Palmer & Driancourt 1981) has the same effect. Practically, 2 hours of light after 9 hours of darkness is recommended. More recently, blue light exposure to one eye via a customised blinker mask (Equilume) has been demonstrated as a practical alternative method of advancing the first ovulation of the year in mares. Photoperiod is not the only determining factor of when a mare begins to cycle. It has been shown that nutrition plays a very important role as well. Mares in good body condition were more likely to cycle all year long, and if they did enter winter anoestrus, they began cycling a month earlier than mares with slightly poorer body condition (Gentry et al. 2002, Waller et al. 2006).

After winter anoestrus, mares go through a period referred to as 'spring transition', characterised by irregular periods of anovulatory follicular waves with or without oestrous behaviour. Spring, or vernal, transition ends when a follicle is induced to ovulate by either a sufficiently large endogenous pulse of luteinising hormone (LH) or an exogenous ovulating agent. In 'late transition', large oestrogenic follicles can develop yet fail to ovulate; these follicles can persist or regress with another follicular wave ultimately ovulating. A somewhat similar period, referred to as the fall (or autumnal) transition, occurs at the end of the breeding season and is similarly characterised by anovulatory follicular waves.

Pharmacological methods to induce earlier cyclicity that have shown promising results involve the use of dopamine antagonists such as sulpiride and domperidone. Results have been variable and appear to be affected by factors such as nutritional status and environmental conditions. Investigations continue with the aim of finding an easy method to induce mares to cycle by the beginning of the breeding season. Even with these methods to induce earlier cyclicity, some mares still undergo a spring transition, a period of variable length that can be frustrating from a breeding management perspective. Mares will develop follicles that reach ovulatory diameter but regress rather than ovulate. On average, 3.7 follicular waves will develop before the first ovulation of the year (Sharp & Davis 1993). A number of ways to shorten spring transition have been suggested, including administration of progestogens, follicular aspiration (Klump et al. 2003), and administration of ovulation inducing agents. Most of the reportedly successful protocols are more effective in late transition than early transition.

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Progesterone/progestogen withdrawal therapy is one option. Progesterone exerts a negative feedback on LH secretion; thus when the source of progesterone is withdrawn or its effect wanes because of the withdrawal of the negative feedback effect, there is an increased release of LH, resulting in follicular maturation and ovulation. Progesterone can be administered as an oil-based intramuscular injection, orally as the synthetic progestogen altrenogest, or by using a silastic progesterone-releasing intravaginal device (PRID; see Chapter 8). The preferred drug for many clinicians is a 10-day course of oral altrenogest at 0.044 mg/kg; however, there are many misconceptions about its use, in particular the mechanisms involved in its action. Altrenogest should, in the authors' opinion, not be used 'blind', i.e., without a prior examination of the mare's reproductive tract with reference to the degree of ovarian activity and the presence of luteal structures on the mare's ovaries. It is debatable whether or not altrenogest advances the first ovulation of the season or rather gives a degree of predictability or synchrony to the late transitional phase. As a guide, altrenogest can be effective but only generally when mares are already into the late transitional phase, characterised by the development of multiple oestrogenic follicles with the largest follicle being larger than 25 mm in diameter. Altrenogest is likely ineffective in mares with minimal follicular activity, particularly during deep anoestrus. The desired response is that the mare either ovulates during the period of treatment or she develops an ovulatory follicular wave after cessation of treatment. If the mare has no mature luteal structures on her ovaries, then the routine use of prostaglandin is unnecessary and inappropriate. One cannot determine what treatment or management plan is appropriate for the mare without knowledge of the degree of follicular development in her ovaries.

Many clinicians in broodmare practice use the intravaginal progesterone releasing devices such as the PRID Delta that, when used in a similar fashion to that described for altrenogest, can be utilised in an attempt to hasten the time to first ovulation. These devices inevitably result in a degree of vaginitis with a variable degree of vaginal discharge, which usually spontaneously resolves post device removal. Occasionally mares may show signs of discomfort and possibly colic when the devices are first inserted. Mares are monitored while the device is in place, and once a follicle reaches a size larger than 36 mm in diameter, the device is removed. Work in the author's (JRC) practice has demonstrated that a single PRID Delta contains enough progesterone to be effective for 10 days in the mare.

In the authors' practices, whether oral progestagen or an intravaginal device is being used, the follicular status of every mare should be examined after 7 days. In mares with follicles measuring larger than 35 mm, supplementation is stopped (or the device removed), and the mare is monitored for ovulation. If the follicles are smaller than 35 mm, supplementation is continued for 3 more days, at which time the treatment is stopped or the device removed. The withdrawal of the negative feedback usually results in oestrus within 6 days and ovulation between 7 and 13 days after the last treatment. By examining the mare when under treatment, the possibility of an unexpected ovulation can be minimised. The use of intramuscular injections of progesterone and oestradiol-17β in oil for 10 days produces a similar response to altrenogest, but the interval to oestrus is longer due to the suppression of follicular development by the oestradiol.

Repeated daily injections of equine pituitary gland extract to mares in winter anoestrus lead to follicular development, although others (Hyland et al. 1987) have reported success using a minipump that infused gonadotrophin-releasing hormone (GnRH) intravenously over a period of 28 days. These last two treatments are impractical for routine use. There have also been reports using GnRH or its analogues, administered by injection, infusion, or subcutaneous implant, to hasten ovulation in transitional or even anoestrous mares (Harrison et al. 1990). It is expensive, as treatment is necessary for at least 1 to 2 weeks, with a mean of 15.8 days (Ginther & Bergfelt 1990). It is noteworthy that these authors found high multiple ovulation rates associated with GnRH treatment.

If a follicle is mature enough and is still in the growth phase, as opposed to being in regression, it will likely to ovulate after treatment with an ovulation agent such as human chorionic gonadotropin (hCG) or deslorelin. This treatment is often used alongside other management or treatment strategies but can be used as a single treatment assuming the mare is in late transition and close to cycling spontaneously.

During the transitional period before the first ovulation of the year, mares demonstrate erratic oestrous behaviour of varying intensity. The presence of multiple large follicles, possibly as large as 30 to 40 mm, can make detection of ovulation difficult by palpation alone. Even outside this transitional period, misinterpretation of ovulation, even by experienced clinicians, has been shown to be as high as 50%. It is much easier to visualise the corpus haemorrhagicum/early corpus luteum ultrasonographically when the anechoic follicle is replaced by an intensely echoic area representing the early corpus luteum (Fig. 31.1A and B). It is recommended that the interval between matings should not exceed 2 or 3 days, although there have been no critical studies on the survival time of sperm in the mares' genital tract. The authors have experienced mares which have conceived after a breeding to ovulation interval of 6 or 7 days, but this is not the norm. An early report in the literature reported pregnancy after a breeding to ovulation intervals of 5.25 to 5.75 days in pony mares (Burkhardt 1949). It is important, however, not to begin breeding too early, or this will result in the mare being mated many times. The appearance of uterine oedema (Fig. 31.2) is considered an indication that the follicle should ovulate within a few days, although some transitional follicles can ovulate in the absence of oedema, and follicles can regress in the presence of oedema. A key factor in the emergence from vernal transition is the development of steroidogenic competence by the follicle, leading to an increase in circulating oestrogen concentrations that causes the release of LH from the pituitary due to a positive feedback mechanism. Oestrogen is responsible for the appearance of uterine oedema (in the absence of progesterone), and so this may be why the detection of uterine oedema is important in signalling the emergence of the mare from the transitional period.

Cystic ovarian disease, comparable to the condition described in the cow (see Chapter 22), does not occur in the mare. The persistent follicles that occur during the transitional and other periods are structurally normal; however, their presence may explain why this condition has been diagnosed in the past.

Abnormal Cyclicity During the Breeding Season

Failure to cycle or the occurrence of irregular cycles during what is considered to be the normal breeding season is another matter. Irregular oestrous behaviour may be observed in a mare in which the pregnancy has failed after the endometrial cups have been established. Endometrial cups, derived from fetal tissue that invades the endometrium at approximately 5 weeks gestation (Fig. 31.3), produce equine chorionic gonadotropin (eCG), which has LH-like activity in the mare (see also Chapter 5). Follicular development continues throughout early pregnancy and eCG causes these mature

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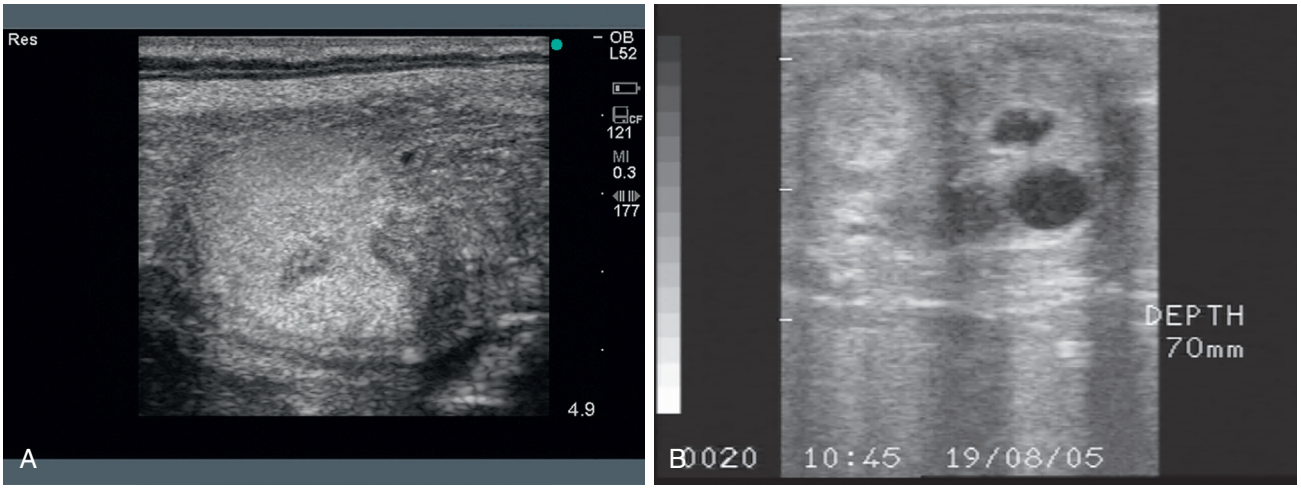

• **Fig. 31.1** Ultrasonographic images of two equine corpora lutea (CL) with a differing ultrasonographic appearances: (A) an early CL that is large and dense (hyperechoic); (B) mature CL with a central lacuna (hypoechoic).

• **Fig. 31.2** Uterine oedema observed by ultrasonography (crosssection of a uterine horn).

• **Fig. 31.3** Endometrial cups present on the endometrial surface of the uterus at approximately 70 days gestation.

follicles to ovulate or luteinise, forming secondary and accessory corpora lutea, respectively (Fig. 31.4). Because of the presence of these follicles and development of additional luteal structures at various times, mares may show brief periods of oestrus but fail to ovulate or may show a prolonged period of anoestrus. This condition will persist until the demise of the endometrial cups (beginning at approximately 80 days gestation) and the disappearance of eCG from the circulation, usually between 100 and 150 days. Occasionally endometrial cups may persist beyond their normal lifespan, resulting in a persistence of eCG in the maternal circulation with consequent effects on ovarian cyclicity (Crabtree et al. 2011).

Rarely, Cushing's syndrome, caused by adenomatous hyperplasia of the intermediate pituitary, has been associated with anoestrus in aged mares. This is presumably due to destruction of the cells secreting luteinising hormone and follicle-stimulating hormone. Mares with equine metabolic syndrome often experience ovulatory failure, and it has been hypothesised that a contributing factor is glucocorticoid production by abdominal fat which inhibits LH release.

'Silent heat' in which a mare is having a normal oestrus and ovulation but fails to show oestrous behaviour, may cause problems when the breeding programme relies on teasing and natural mating, but with the use of ultrasonography and artificial insemination, this is more easily remedied. The incidence of silent oestrus has been reported to be 6% (Nelson et al. 1985). Silent oestrus is more common in mares with a young foal by their side and in maiden mares early in the breeding season. Other factors affecting oestrous behaviour include being kept with very dominant mares and stallion preferences. Fillies that are in training and have been treated with anabolic steroids may be more likely to suffer from this due to 'androgenisation'.

Ultrasonography and vaginal examinations can confirm that the mare is in oestrus and has follicles of an ovulatory size. It

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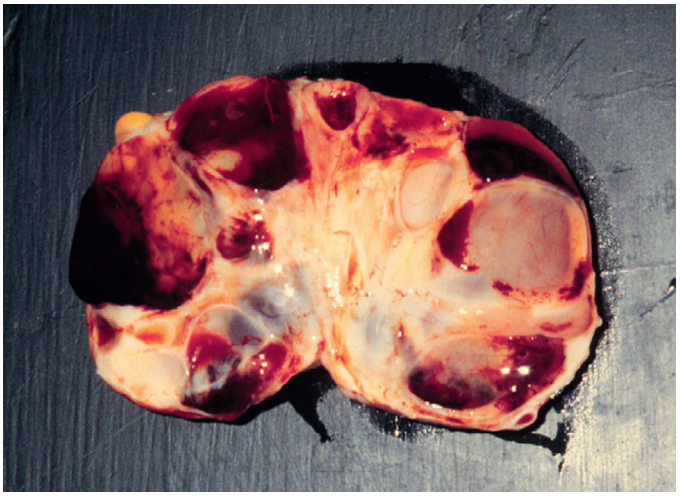

• **Fig. 31.4** Multiple follicles and luteal structures on the ovaries of a mare 60 days pregnant.

is essential to distinguish the condition from a prolonged luteal phase, in which there is follicular development but a persisting luteal structure secreting progesterone. The use of exogenous PGF is recommended if any doubt exists about the presence of luteal tissue.

Treatment is based on thorough and careful teasing. Frequent and persistent teasing may persuade the mare to show oestrus. Management options include safely restraining the foal within sight of the mare, removing the foal out of auditory range, and tranquilising the mare. Alternatively, placing the mare in a stable next to a stallion may be helpful. If permissible, artificial insemination can be used. In order to breed mares naturally during a silent oestrus, some form of restraint may be necessary; many mares approaching ovulation accept the stallion when twitched and hobbled. An intramuscular injection of oestradiol benzoate (10–20 mg) 6 hours before breeding can be tried as a last resort; however, this drug is no longer available in many parts of the world. The veterinary surgeon must ensure that the mare is physiologically ready to be bred. In some cases when the mare is not psychologically prepared for breeding, treatments are of little value, and although tranquillisation may allow the mare to be covered, pregnancy is unlikely to be achieved. In many cases, it is a failure of oestrous detection rather than a true reproductive disorder of individual mares. However, it has been associated with reduced oestradiol concentrations in the peripheral circulation and a shorter interval from luteolysis to ovulation (Nelson et al. 1985). There is no suggestion that aberrant morphological abnormalities in follicular development are involved.

Prolonged Luteal Activity

Persistence of luteal activity in the nonpregnant mare is a major cause of subfertility. Traditionally, the term 'prolonged dioestrus' has been used to describe a condition in which the corpus luteum (CL) persists beyond its normal cyclical life span of 15 to 16 days, resulting in the maintenance of elevated circulating progesterone concentrations for longer than expected. Ginther (1990) has suggested that the term 'prolonged luteal activity' should be used, as 'persistent dioestrus' implies that the CL persists, whereas it is possible that others are formed sequentially from dioestrous ovulations. In mares, although progesterone inhibits follicular development, it does not inhibit it completely. Therefore mares continue to develop medium to large follicles during dioestrus. Usually, these follicles undergo atresia. However, occasionally, a large follicle will ovulate during dioestrus. These occur in up to 20% of oestrous cycles in Thoroughbred mares (less frequently in some other breeds and ponies) and are not accompanied by oestrus; the cervix will remain pale in colour, dry, and tightly closed. If a dioestrous ovulation occurs late in the luteal phase, when PGF is released from the uterus and lyses the original CL, the more recent CL is at too early a stage in development to respond to the PGF. It then continues to develop, produces progesterone, and prolongs dioestrus.

True persistence of the CL occurs in approximately 20% of ovulations. These mares are a problem for the stud manager, as they can be assumed incorrectly to be pregnant. With stud farm management practices of today, a persistent CL or dioestrous ovulation are easily diagnosed and treated. Plasma progesterone profiles are indistinguishable from those of pregnant animals. The uterus becomes firm and tubular (toned), and the cervix is typical of that of pregnancy; however, transrectal ultrasound imaging fails to detect a conceptus confirming nonpregnancy.

Failure of synthesis and/or release of $PGF_{2\alpha}$ at the end of dioestrus is the most likely cause of persistence of the primary CL. Ginther (1990) has suggested that it might also be due to failure of the CL to respond to PGF, or failure of PGF to reach the CL. Treatment is by the injection of a luteolytic dose of PGF or a synthetic analogue. The interval between treatment and ovulation varies considerably depending on the size of follicles at the time of treatment. Therefore it is advisable always to examine mares using ultrasonography before treatment in order to assess the status of follicular development.

The term 'pseudopregnancy' is often used in the case of a mare that was bred, subsequently has a prolonged luteal period, and is not pregnant when examination is performed. It is then assumed that an embryo was present at the time of maternal recognition of pregnancy but was subsequently lost before the time of pregnancy examination. With the capability to diagnose pregnancy at day 10 to 12, before the time of maternal recognition of pregnancy, using the higher quality ultrasound machines currently available, the true incidence of pseudopregnancy should be readily discernible. True pseudopregnancy occurs if there is early embryonic death after 15 days of gestation, with persistence of the primary CL resulting in a prolonged luteal phase. The cervix remains tightly closed, and the uterus is toned and tubular. It is diagnosed based on an early positive pregnancy examination, followed by a negative pregnancy examination on ultrasound examination during a maintained luteal phase.

Seasonal Anoestrus

Seasonal anoestrus is the term that best describes the mare that has ovarian inactivity (anoestrus) during the normal physiological breeding season. The most commonly encountered form of seasonal anoestrus is that encountered in lactating mares after foaling and is often referred to as 'lactational anoestrus'. Affected mares may have a normal postpartum oestrus (foal heat) after 6 to 12 days but fail to return to oestrus at the end of the first dioestrus. Alternatively, they may not have a normal foal heat at all. This seems to be more common in mares that foal far outside the normal physiological foaling/breeding season. It also appears to be related to nutrition because mares in good bodily condition are less likely to experience seasonal anestrus than those in poor bodily condition. The ovaries resemble those of a mare in deep winter anoestrus, i.e., small and inactive, and the condition can last for several months. Originally it was thought to be due to the presence of high levels

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of prolactin suppressing pituitary gonadotrophin release, but this is now in doubt. Affected mares should be teased and examined weekly per rectum to assess their ovarian status. It is expected that most mares will have a 'foal heat' and ovulate at 7 to 14 days post-foaling; mares with little or no ovarian activity at this stage often necessitate treatment to avoid a prolonged period of anoestrus.

In the authors' experience, the preferred treatment for this would be domperidone at 1.1 mg/kg orally once daily; if the mare fails to respond with the development of a follicle of preovulatory size in 7 to 10 days, then additional therapy with progestagen or GnRH should be used. Alternatively, the GnRH analogue buserelin (Veterelin, Receptal), 20 to 40 mcg twice daily by intramuscular injection for up to 10 days or with consideration to the prescribing cascade, a human GnRH analogue Goserelin, available in a 3.6 mg implant (Zoladex), can be used. Half an implant (1.8 mg) is inserted under the skin of the mare, and a response is usually seen within 7 to 10 days. Treatment with GnRH is often expensive and the pregnancy rate at the induced oestrus is perceived to be reduced. In addition, the mare may return to anoestrus after the induced ovulation.

Seasonal anoestrus in young, nonfoaling mares that are in good physical health and condition presents a more difficult challenge. Sexual immaturity and karyotypic abnormalities leading to gonadal dysgenesis are two potential factors to consider; however, severe malnutrition, advanced age, and mares treated with anabolic steroids may also have abnormally small and inactive ovaries. Some presumed 'normal' mares can encounter prolonged periods of acyclicity. Allowing the mare additional time is often the first approach, but if after a period of several weeks during the physiological breeding season the mare has not developed any significant sized follicles or evidence of follicular waves, further investigations or attempts at therapy can be made. The clinician must first determine that the mare is not in a prolonged luteal phase (persistent dioestrus); if there is any doubt, a progesterone assay can be performed to clarify whether there is any active luteal tissue present. Assays for FSH and LH can be performed, along with GnRH challenge to determine whether the hypothalamic–pituitary axis is functional; however, this requires access to a specialist laboratory able to perform them. Treatment with progestagens and/or domperidone is generally used first, but if the mare shows little or no response to these, then GnRH analogues can be used.

A differential cause of a mare or filly with apparent seasonal anoestrus or a failure of follicular development would be previous vaccination against GnRH. Such procedures have become popular in competition horses for the suppression of unwanted sexual behaviours. A GnRH vaccine is commercially available in Australasia (Equity oestrous control vaccine, Zoetis, Australia) for the suppression of oestrus and oestrous-related behaviour in mares not intended for breeding. Such vaccines are also available for other species, such as pigs, and although Equity is only available in Australasia, vaccines for other species are being used 'off-label' in mares in other parts of the world. Mares cease ovarian activity within approximately 4 weeks of the second injection of a primary course and remain suppressed for a variable amount of time (Imboden et al. 2006, Elhay et al. 2007, Schulman et al. 2013). The younger the mare is when vaccinated, the longer the interval to reversibility is likely to be (Schulman et al. 2013). In a study by Imboden and co-workers (2006) of nine mares vaccinated twice, five returned to ovulatory activity in 28 to 100 weeks, three resumed follicular activity without ovulating, and one remained inactive over the 100 week observation period. They concluded that the duration of the inhibitory effect is highly variable, not clearly related to GnRH antibody titres, and resumption of ovulatory activity may require more than 100 weeks. In addition, ovarian suppression did not always prevent the occurrence of sexual behaviour (Imboden et al. 2006), and this would create a very difficult situation if it was not known that the mare had been vaccinated. It is not known for how long mares that receive repeated doses of vaccine (boosters) will remain reproductively inactive.

Genetic Abnormalities

Genetic abnormalities must be considered in a mare that fails to cycle. Although not common, many types of sex chromosome anomalies have been described in the horse. The incidence of chromosomal abnormalities is difficult to assess but must be suspected in maiden mares with very small (< 1 cm), inactive ovaries and an immature tubular genital tract once winter anoestrus has been eliminated as a cause of acyclicity. However, some genetically normal young fillies in training can be acyclic, and thus they must be given more time to mature reproductively. A thorough history and physical examination are necessary. Depending on the particular genetic abnormality, the reproductive tract can be rudimentary, juvenile, or ambiguous. The 63 XO, X chromosome monosomy, often referred to as equine Turner's syndrome (gonadal dysgenesis), is the most common chromosome abnormality in horses with a 'normal' external female phenotype (Blue et al. 1978, Kutzler 2001). This can be confirmed using cytogenetic analysis to determine the karyotype of the individual. Other chromosome abnormalities are rare but include 63 XO/64 XX mosaics, 64 XY sex reversals and 65 XXX trisomy (Blue et al. 1978, Nie et al. 1993, Kutzler 2001, Vallagómez et al. 2011). Most of the XY sex reversal cases are Y chromosome positive but SRY-gene negative. The deletion of the SRY gene causes the fetus to 'default' to the female developmental pathway. Another genotype of 64 XY, SRY positive with female phenotype has been described, which may indicate that other gene mutations are involved (Vallagómez et al. 2011).

Multiple Ovulation and Twinning

Double ovulations occur in up to 30% of oestrous cycles, the frequency depending on the breed and type of the mare (e.g., Thoroughbreds have a higher rate than ponies). Accurate detection of such ovulations is important, as twinning is highly undesirable: first, because it often results in abortion (Fig. 31.5), and second, even if both fetuses survive and are carried to term, many are dysmature, resulting in a high neonatal mortality rate and dystocia with a potentially serious effect on future fertility of the dam, is very likely. A further complication is that if embryonic/fetal death occurs after the formation of the endometrial cups (see Chapters 4 and 5), these latter structures persist until they spontaneously regress as if pregnancy had been maintained, thus resulting in pseudopregnancy.

Not all conceived twins persist as after fixation, unilateral twins will be naturally reduced to a singleton 85% of the time (Ginther 1989a,b). Ginther (1989c) hypothesised that the mechanism for this was via deprivation of adequate embryonal–maternal exchange. Natural reduction via the 'deprivation hypothesis' occurs when a major portion of the vascularised (trilaminar) wall of one member of a twinset is apposed to the wall of the adjacent twin rather than with the endometrium (Ginther 1989c). In physical terms the thick vascularised portion of the small vesicle is forced by the myometrial tone and contractions into the thinner nonvascularised portion of the large vesicle, and therefore the smaller vesicle is unable to obtain adequate nutritional support from the endometrium and fails to survive (Ginther 1998). This explains the reason why, when

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• **Fig. 31.5** Aborted twin fetuses.

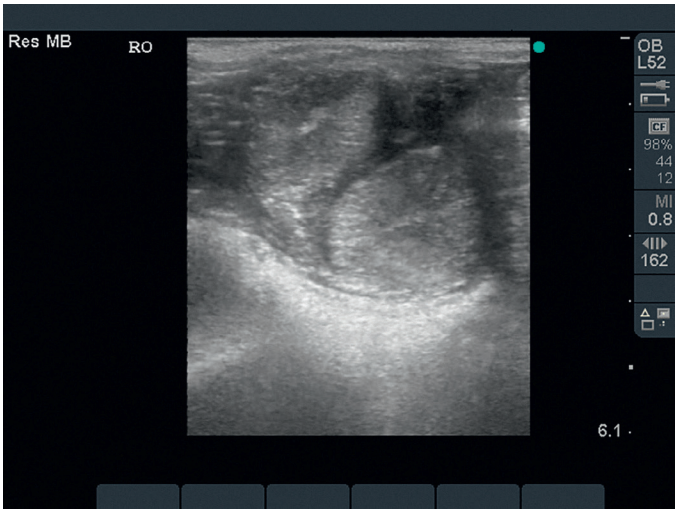
twins of dissimilar size (greater than or equal to 4 mm) fixate unilaterally, the vast majority naturally reduce to a singleton with regression of the smaller vesicle. When each member of the twinset is of similar size (< 3 mm difference), 27% will persist as twins (Ginther 1984, Ginther 1989c). It is the authors' experience that natural reduction is generally complete by day 35 of gestation and is usually preceded by changes in the heart rate or Doppler signal of the dying member of the twinset. Natural twin reduction does not generally occur before day 15 and does not occur when twins fixate bilaterally; therefore twin pregnancies that are detected during the mobility phase or are bilaterally fixated are best managed by the manual transrectal crushing of one embryonic vesicle.

Rectal palpation alone can be misleading in detecting a double ovulation, particularly when the two follicles are on the same ovary. The use of ultrasound examination of the ovaries, which should routinely be performed in conjunction with a thorough transrectal palpation, usually allows detection of a double ovulation. Sometimes the ovulatory area can appear indistinct for the first 24 hours; in these cases the mare should be reexamined 2 days later when it can be seen more easily whether there is more than one CL (Fig. 31.6).

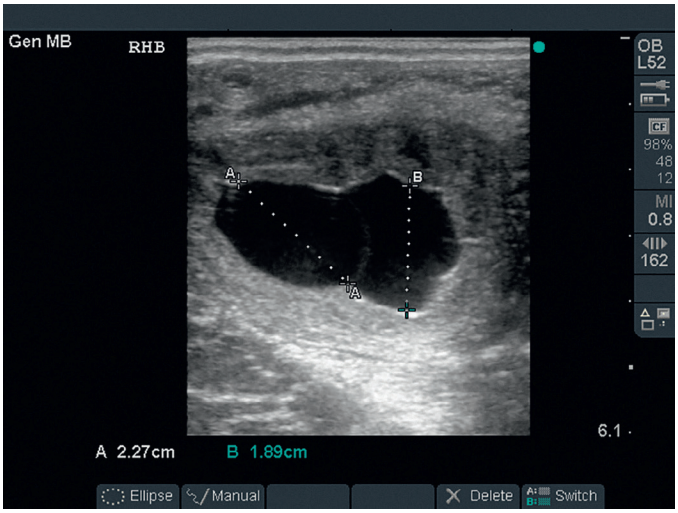
Multiple ovulations in the mare should not be regarded as a reason for withholding breeding. Instead, pregnancy rates are improved after twin ovulation. Although accurate interpretation of the ultrasound images of early pregnancies in the mare and the technique of crushing a conceptus are skills that require experience, the advent of B-mode ultrasound imaging has provided a method of more readily managing a twin pregnancy in the mare (Fig. 31.7).

Approaches to dealing with twins, depends on the stage of gestation at the time of detection:

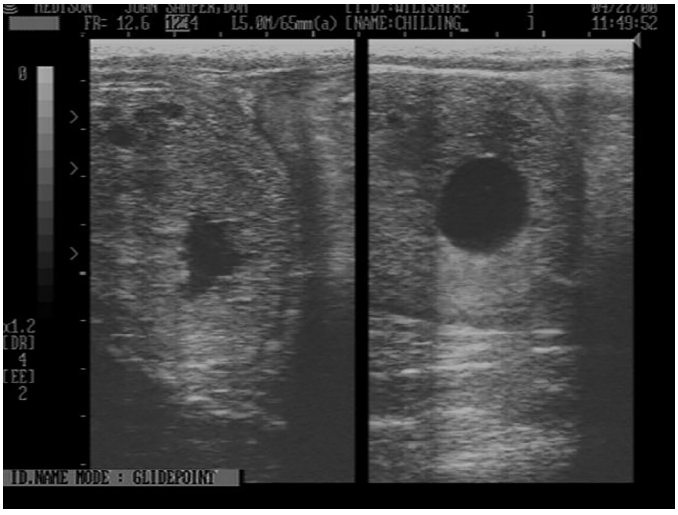
1. If the initial examination of the mare occurs before fixation (day 16–17), the twin embryos are reduced to a singleton by the manual destruction of one, either by pressure with the transducer or by the use of the hand (Fig. 31.8). One of the vesicles is isolated and crushed using the tips of the fingers and thumb, the fingers against the palm of the hand, or with pressure using the transducer.



• **Fig. 31.6** Two mature corpora lutea on the same ovary, leading to twin pregnancy.



• **Fig. 31.7** Unilateral twin conceptuses, 16 days of gestation.



• **Fig. 31.8** Ultrasonographic appearance after crushing one of two twin vesicles.

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During the mobility phase, the conceptus vesicles can be separated gently using the transducer to enable the procedure to be imaged. Alternatively, because the vesicles are still in the mobility phase, if the vesicles are situated next to each other, the mare can be reexamined after a short time interval and in many cases the vesicles will have moved apart spontaneously, facilitating isolation of a single vesicle. When the conceptus vesicles are of dissimilar sizes, the smaller one should be ruptured; this is easier at days 14 to 16 when they are 14 to 20 mm in diameter than days 11 to 13 when they are 6 to 11 mm in diameter.

Prefixation manual reduction of twins has the greatest success rate of producing a single normal healthy foal, and does not increase the risk of pregnancy loss higher than those associated with singleton pregnancies (Davies-Morel et al. 2012.) However, mares older than 9 years of age that have a twin reduced are potentially at greater risk of pregnancy failure (Schnobirch et al. 2013). In our veterinary practice (JRC), the subsequent pregnancy failure rate for the remaining member of a twinset is lower than that of singleton pregnancies. Success is dependent on operator experience but is not generally influenced by the position of the twins in the uterus (Sheerin et al. 2010, Schnobirch et al. 2013). Treatment with flunixin meglumine and altrenogest after reduction may reduce pregnancy failure rates by up to 5% (Sheerin et al. 2010) but should be applied on a case by case basis.

The disadvantage of this method is that it is more expensive, in that all mares are scanned before the time of return to oestrus. In addition, if ovulations that occur more than 3 days apart have not been detected, a mistaken diagnosis of a single pregnancy may be made if the second vesicle is too small to detect.

2. If initial examination is done after fixation but before day 30 and if the conceptuses are in opposite horns (bilateral twins), then it is best to manually crush them immediately up to 30 days, as this is usually effective in successfully reducing to a singleton. If both conceptuses are in one horn (unilateral twins), manual reduction can be attempted as long as the vesicles retain their spherical shape and can be separated; as soon as the vesicles coalesce and are separated only by a line on the ultrasound, manual reduction should not be attempted. Another option is to terminate the pregnancy using $PGF_{2\alpha}$. An additional approach is to use transvaginal ultrasound-guided allantocentesis of one vesicle.

Management of twin pregnancies after this period is complicated by the formation of endometrial cups at approximately day 35 of gestation. Endometrial cups remain functional until around days 100 to 150 of gestation in the presence or absence of a viable fetus. Therefore if twin pregnancies are not successfully managed before the cups are formed or if both embryos die after day 35, the mare usually will not return to a fertile oestrus for a prolonged period of time.

3. As twin pregnancy is allowed to progress further in gestation, the management options are reduced and success rates become lower. Dietary restrictions are commonly referred to as a means of reducing a twin pregnancy to a singleton. The evidence for this originates from one study performed by Merkt and co-workers (1982), who reported a 63% chance of success in reducing a twin to a singleton with dietary restriction for 2 to 4 weeks. It must be noted, however, that they also reported a 17% abortion and a 20% pregnancy failure rate; in addition this study was performed postfixation (from 21 days) and in the absence of any ultrasound examinations it is unknown to what extent natural reduction occurred irrespective of the effect of dietary restriction. As a result, such an approach cannot be recommended.

Management options include: manual reduction techniques, transvaginal ultrasound-guided twin puncture, cranio–cervical dislocation, and transcutaneous ultrasound-guided twin reduction. The option for repeated manual traumatisation (membrane slip/pinching/ballottement) still exists; however, there are some novel manipulation techniques available. One technique, described by McKinnon (2011), involves the oscillation of the fetus on its umbilical cord resulting in its dislocation from the umbilical cord. Transvaginal ultrasound-guided needle puncture involves the puncture of the conceptus with aspiration of the yolk sac or allantoic fluid, with or without stabbing the fetus. This is done via a transvaginal approach using a specifically designed transvaginal convex ultrasound probe and needle guide, with a suitably long aspiration needle. This is a technically demanding procedure requiring specialised and inevitably expensive equipment; as such, it is only performed by a limited number of specialists. The technique is successful in 33% of cases (Journée et al. 2013). Two different cranio–cervical dislocation (decapitation) techniques have been described (Wolfsdorf et al. 2005, 2009). The first is cranio–cervical dislocation of one member of a twinset via a transrectal approach between 55 and 90 days of gestation. The second is via a standing flank laparotomy between 58 and 110 days of gestation. Both techniques offer a 63% chance of producing a healthy single foal. Transcutaneous ultrasound-guided twin reduction is best performed between 115 and 130 days of gestation, and it involves the killing of the least developed member of a twinset by transcutaneous intracardiac injection of potassium chloride (Macpherson & Reimer 2000) or injection of the fetal heart, lungs, or abdomen with 10 to 20 mL of procaine penicillin (Ball 2005). After fetal death, the fetus gradually becomes mummified. Success as determined by the overall percentage of live singleton foals born ranges from 38% to 56% (Macpherson & Reimer 2000, Ball 2005, McKinnon 2011).

Failure to Establish and Maintain Pregnancy

Pregnancy failure is a source of major economic loss to the horse industry. Embryonic death occurs before organogenesis is complete, at approximately 40 days of gestation in horses. Abortion is defined as expulsion of the fetus before it is capable of life outside the womb, whereas a stillbirth is expulsion of a dead fetus that would have been capable of life outside the womb.

Embryonic Death

Fertilisation rates are normally high in horses, although differences may exist between normal and subfertile mares. Pregnancy losses are greatest during the embryonic period, estimated to be as high as 24% in mares (as reviewed in Ball (1988)). The period of greatest embryonic death in subfertile mares occurs in the interval before pregnancy can be detected with ultrasound (approximately day 11), particularly at the time when the embryo enters the uterus. Between days 14 and 40, estimates of the rate of embryonic death vary between 8% and 17%. Predicting embryonic loss can be difficult.

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Examination by transrectal ultrasonography provides the best means to predict and detect embryonic loss. Because pregnancy is often diagnosed at an early stage, it is important to inform owners that not all pregnancies detected with ultrasound will survive, even when apparently normal. Embryonic vesicles found to be smaller than expected for their age are considered to be at increased risk, whereas oversized vesicles are not (Ginther et al. 1985). Embryonic loss may occur between days 11 to 15 postovulation, without any ultrasonographic indication of its impending occurrence. Later in gestation, however, indications of impending loss may be observed and include a speckled or granular appearance of the conceptus, mobility of the vesicle after the normal time of fixation, fluid surrounding the vesicle, absence of a heartbeat, decreased volume of the conceptus, disruption of conceptus membranes, or oedema of the endometrial folds (Ginther et al. 1985).

In many species, genetic abnormalities play a significant role in pregnancy loss. Chromosomal abnormalities have been identified in normal appearing equine embryos (Rambags et al. 2005), and an increased incidence of embryonic loss has been associated with certain family lines or stallions. Many chromosomal abnormalities may not be inherited but may arise during formation and ageing of gametes and result in an inability of the resulting zygote to develop into a viable embryo. Ageing gametes may be increased when mating is not closely timed to that of ovulation. The incidence of embryonic loss is higher when insemination occurs after ovulation, and increases as the interval from ovulation to insemination lengthens (Koskinen et al. 1990).

A critical period occurs at 14 to 16 days of gestation when the presence of a conceptus inhibits luteolysis. Factors of conceptus origin inhibit the release of PGF from the endometrium and are essential for the maintenance of luteal function (Stout & Allen 2002). Unrestricted conceptus mobility is essential for pregnancy maintenance and prevention of luteolysis (Sharp et al. 1989). Anything that interferes with embryonic mobility, such as transluminal adhesions or endometrial cysts, or with uterine contractions that are the main propulsive force stimulating embryo movement may result in embryonic loss.

Extremes of age, either young or old, negatively affect fertility. The high rate of embryonic loss in yearling mares has been attributed to immaturity, inadequate nutrition, or physical stress (Mitchell & Allen 1975). Older mares have a lower pregnancy rate and a higher loss rate compared with younger mares (Ball et al. 1989). The effect of advanced age, however, is difficult to separate from acquired subfertility and is confounded by the effect of parity as the two are generally correlated.

The effect of age on fertility, at least in part, originates in the oocyte. When embryos were collected from the oviducts of young mares and old mares, significantly fewer cells and poorer morphology were observed in embryos from old mares (Carnevale et al. 1993). In a subsequent study (Carnevale & Ginther 1995), oocyte transfer was used to compare fertility of oocytes from young mares and old mares, and more embryonic vesicles resulted from young than old mares. Significantly fewer oocytes or embryos were recovered from mares 20 years of age or older than from mares 10 years of age or less, suggesting failure of ovulation or oviductal harvesting of the oocyte. Consequently, fewer oocytes had the potential for fertilisation and embryo development in the older mares (Carnevale et al. 1993). Other researchers have similarly found a lower pregnancy rate and an increased incidence of early embryonic death after transfer of embryos from subfertile mares compared with those obtained from maiden mares (Iuliano & Squires 1986, Vogelsang & Vogelsang 1989).

Oviductal pathology may result in either embryonic loss or fertilisation failure. Uterine pathology may affect nutrient transfer to the early embryo via histotrophe production, and result in retarded embryonic development or embryonic death.

Progesterone is essential for the maintenance of pregnancy. The only source of progesterone during the embryonic period is the primary CL (corpus luteum verum). To the authors' knowledge, there is no published evidence that progestagen supplementation will improve conception or 14 day pregnancy rates, yet anecdotally, it is used in cases of repeated conception failure. On the assumption that luteal insufficiency is important in early embryonic death (EED), many mares are given exogenous progesterone or progestogens in an attempt to prevent it from occurring. However, the rationale for this widespread practice is questionable. Although primary luteal insufficiency as a cause of EED has been reported (Bergfelt et al. 1992, Canisso et al. 2013), little evidence exists to indicate that primary luteal insufficiency is a major cause of embryonic loss; in fact, pregnancy loss is rarely caused by a fall in plasma progesterone (Irvine et al. 1990). Progesterone supplementation has been reviewed by Allen (1993), who is sceptical of any benefit. Nevertheless, withdrawal of supplementary progesterone therapy during midgestation may leave the clinician open to criticism if the mare subsequently aborts. Progesterone therapy is appropriate in mares that have uterine oedema and an indistinct corpus luteum at the time of first examination for pregnancy (15 days). These pregnancies are usually lost within a few days, but some can be successfully 'saved' by exogenous progesterone with the pregnancy maintained to term (Canisso et al. 2013).

Secondary luteal insufficiency, resulting from uterine pathology or unrelated inflammatory conditions, is a more likely cause of embryonic loss (Daels et al. 1989, Stabenfeldt & Hughes 1987). Inflammatory conditions with associated PGF release, even soon after ovulation and especially before endometrial cup formation, warrant attention. PGF inhibitors are effective in blocking PGF release but must be administered soon after the inciting cause (Daels et al. 1989). A more likely approach involves administration of progestogens. Exogenous progestogen can maintain pregnancy even after luteal activity is compromised (Daels et al. 1989) and should be continued until placental progestins are capable of maintaining pregnancy by around 100 days of gestation. Supplemental progesterone, however, has not improved pregnancy rates in normal mares receiving embryos (Iuliano & Squires 1986). Many dosage regimens do not effectively elevate or maintain plasma progestogen levels. Progesterone in oil (100 mg, IM), altrenogest **X** (0.044 mg/kg, by mouth) given daily, or a long-acting formulation of progesterone (BioRelease P4, BET Pharm) given weekly have been shown to be able to maintain pregnancy in ovariectomised mares and are therefore considered adequate. A number of synthetic progestogens, including medroxyprogesterone, norgestomet, and megestrol acetate, were unable to maintain pregnancy (McKinnon et al. 2000).

If altrenogest is to be administered to the mare with conception failure the authors would suggest that it is administered from no earlier than day 5 postovulation. If the mare becomes pregnant, therapy should be continued until either it can be demonstrated that endogenous progesterone levels are adequate (> 3 ng/mL; assay dependent) because the mare has secondary ovulation(s) that will boost endogenous progesterone production or until the fetoplacental unit takes over progesterone production at around 100 days of gestation. The authors recommend that mares are weaned off altrenogest by halving the dose for at least 1 week.

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There is a general notion that altrenogest therapy will do no harm. However, it should be recognised that altrenogest may suppress endogenous progesterone levels. A study by DeLuca and co-workers (2011) demonstrated that administration of altrenogest to pregnant mares was associated with lower concentrations of endogenous progesterone from days 14 to 18 and on day 21, compared with endogenous progesterone levels in pregnant mares not treated with altrenogest. This effect was presumed by these authors to be mediated by a reduction in pituitary LH release and a decrease in luteotropic support.

To ensure pregnancy maintenance, effective exogenous progestogen therapy should be continued through the first 4 months of gestation, until the placenta can be considered to provide adequate progestogens for pregnancy support. It is noteworthy that if using exogenous altrenogest, plasma progesterone concentrations can still be measured accurately because altrenogest does not cross react with the assay. When plasma progesterone is greater than 3 ng/mL (assay dependent), the altenogest can be discontinued. If the uterine environment is normal, progesterone therapy is probably not needed (Stabenfeldt & Hughes 1987); however, the difficulty lies in determining whether the uterine environment is normal from the viewpoint of the conceptus. Viability of a pregnancy should be reaffirmed when long term progestogen therapy is practised. Retention of a conceptus after fetal death may result from continued treatment.

Field trials conducted by Newcombe and co-workers (2001) on 2346 mares, determined that treatment with 20 to 40 μg buserelin between Days 8 and 12 significantly increased pregnancy rates by approximately 10%. Given the timing of this therapy, the effect is not via improved conception rates, but rather by decreasing pregnancy loss before 14 days of gestation (Pycock & Newcombe 1996b). The mechanism for this remains to be determined; however, increases in circulating LH concentrations have been demonstrated in buserelin treated mares (Kanitz et al. 2007).

Endometritis, which is often the result of a delay in uterine clearance of bacteria, is an important cause of embryonic loss in mares (Woods et al. 1987) and usually causes loss before maternal recognition of pregnancy. Once pregnancy is established, endometritis is a less frequent cause of embryonic loss (Ricketts 2003), provided anatomical defects are corrected. Endometritis is described in more detail elsewhere in this chapter.

Some specific infectious diseases have been associated with embryonic loss. For example, contagious equine metritis (CEM), a transmissible venereal disease caused by *Taylorella equigenitalis,* commonly results in embryonic loss after initial infection (Timoney & Powell 1988). Asymptomatic carriers of both sexes allow persistence within the horse population. Carrier mares, which may or may not have shown signs of previous endometritis, harbour the organisms in the vestibular area, particularly the clitoral fossa and sinuses. To test a mare for CEM, the medial clitoral sinuses (Fig. 31.9) should be swabbed and then seeded on Amies charcoal medium to be transported (kept preferably at 4°C) to a diagnostic laboratory. Information regarding control procedures for CEM and the other venereal pathogens are in national governmental or breed association guidelines and regulations.

Mare reproductive loss syndrome (MRLS), a problem associated with eastern tent caterpillars in Kentucky, is another specific syndrome associated with EED. Although much remains to be learned about the syndrome, it appears that ingestion of the setae of the caterpillars is involved. Reproductive losses occur in the first and last trimester (Riddle 2003). Nonreproductive syndromes associated with the disease include pericarditis and endophthalmitis.

• **Fig. 31.9** Swabbing the medial clitoral sinus for the contagious equine metritis (CEM) organism.

Mares normally resume cyclical ovarian activity very shortly after parturition, so that they are sometimes bred as early as 7 to 10 days postpartum (at the foal heat). There is conflicting evidence about the level of embryonic death if fertilisation occurs at this time, with some studies showing a higher rate and others no effect. An advantage of breeding at the first oestrus postpartum is that the foaling date will be approximately 1 month earlier the next year. Therefore the later a mare foals, the more pressure there is to breed at the foal heat. Pregnancy rates are clearly influenced by the selection criteria for mating at the foal heat. Some studies have found that even though pregnancy rates may be lower, pregnancy losses are no greater after foal heat breeding than after later breedings (Woods et al. 1987, Lowis & Hyland 1991). The presence of intrauterine fluid or ovulation before 9 days postpartum are correlated with lower pregnancy rates and a greater risk of embryonic loss; therefore an ultrasound examination of mares before foal heat breeding will help to differentiate those mares that are good candidates for breeding from those in which breeding should be postponed.

Maternal stress due to severe pain, malnutrition, or transport has been implicated as a cause of EED. Nutritional stress, either from malnutrition (van Niekerk 1965) or poor quality protein in the ration (van Niekerk & van Niekerk 1998), has been associated with an increased incidence of pregnancy loss. A good nutritional programme is clearly advisable for pregnant mares. Other stresses, such as hauling for long periods, are often suggested as causing embryonic loss. However, controlled studies failed to substantiate this belief (Baucus et al. 1990), and recent work failed to demonstrate any difference in pregnancy rates between transported and nontransported mares. The common practice of transporting mares to stud for mating and returning home the same day should not be detrimental to their fertility as long as the transport is safe and comfortable. Far from being avoided, regular exercise is important during pregnancy, although during the latter half, forced exercise

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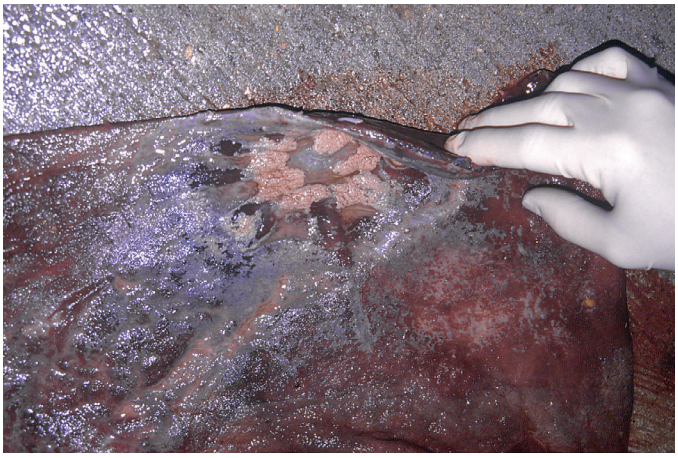
• **Fig. 31.10** Cervical star on the chorionic surface of the equine placenta.



• **Fig. 31.11** Placentitis on the chorionic surface; note the discoloured area.

• **Fig. 31.12** Twin placentas; notice avillous areas where the placentas were in contact.

should be decreased. Rectal palpation and ultrasound examinations are safe procedures when performed correctly, and there is no indication that ultrasound examination is detrimental to the embryo (Vogelsang et al. 1989).

Abortion

An overall abortion rate of 10% after 60 days of gestation is usually cited as being normal for the horse; in practice, it is important to distinguish infectious from noninfectious causes. Vaginal discharge, premature lactation, and colic in pregnant mares may indicate an impending or recent abortion.

When abortion occurs, the mare should be isolated, a history obtained, and the fetus sent to an approved laboratory for necropsy. If a veterinary surgeon wishes to perform a postmortem examination, small but representative samples of liver, lung, thymus, spleen, and chorioallantois (ideally multiple sites, one of which is from the cervical star, which is the irregular, star-shaped avillous area of the chorion that lies over the internal os of the cervix (Fig. 31.10) (see also Chapter 4) should be sent fixed in formalin for histological examination. In addition, fresh (unfixed) tissues for equine herpesvirus 1 and 4 detection (EHV 1, 4) by polymerase chain reaction (PCR) should also be submitted. Paired serum samples from the mare and close companions can also be taken for serological investigation. Swabs from fetal heart/pleural fluid or liver/peritoneal fluid and the cervical pole of the chorion are used to screen for bacterial infection.

The fetus and fetal membranes (amnion, chorioallantois, and umbilical cord) must be carefully examined for the presence of abnormalities and areas of discolouration (Fig. 31.11). Placental evaluation in the field has been described (Cottrill et al. 1991, Rossdale & Ricketts 2002, Morresey 2004, Schlafer 2004), and a detailed description of abortion in mares has been described by Acland (1993). Many countries have regulations or codes of practice for the general approach to the management of an aborting mare, and these should always be followed.

Noninfectious Causes of Abortion and Stillbirth

Historically, twins have been the single most important cause of abortion in Thoroughbreds. However, they are now much less common due to the widespread use of ultrasonography. The diagnosis of twin pregnancy abortion can be made even if only one fetus is found, as examination of the placenta reveals an area devoid of villi where the two placentas (Fig. 31.12) were in contact. Twins should still be submitted to a diagnostic laboratory as twin pregnancies are equally susceptible to infectious abortion with EHV 1, 4.

In mares the umbilical cord is twisted, usually in a clockwise spiral. The normal length ranges from 36 to 83 cm (mean 55 cm). Increased cord length (over 80 cm total length) has been associated with excessive cord torsion. This twisting results in increased resistance to blood flow in both directions, and the resulting thrombosis and poor placental perfusion can lead to fetal death and abortion of an autolysed fetus. Decreased cord length can cause premature tearing of fetal membranes, leading to fetal asphyxia. Twisting and vascular compromise reportedly constitute the most common single cause of observed noninfectious abortion in the UK (Smith et al. 2003).

In mares, the interdigitating microvilli are connected by an unidentified electron-dense material. Placental separation involves dissolution of this substance and can occur prematurely. Causes of premature placental separation are largely unknown, although maternal stress and endophyte-contaminated tall fescue have been implicated. When placental separation occurs shortly before parturition, the thickened placenta often does not rupture through the cervical star, and the allantochorion bulges from the vulva as a so-called 'redbag' delivery (Fig. 31.13). As the placenta is

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• **Fig. 31.13** Premature separation of the placenta, commonly called 'red bag' delivery.

separating, the foal can become hypoxic, resulting in neonatal maladjustment syndrome.

Normal equine pregnancy is situated primarily at the base of a uterine horn, resulting in the pregnancy being positioned in the body and a pregnant horn. On occasion, the pregnancy fixates and develops in the uterine body. In this condition almost the entire chorionic surface of the placenta contained within the uterine body is without villi, whereas that contained within the horns is covered with an excessive number of villi. The proportion of the placenta corresponding to the two uterine horns is small, and the fetus is situated entirely within the uterine body. The fetus is growth retarded and is frequently aborted completely contained within its placenta. The abortion occurs when the nutritional demands of the fetus exceed the ability of the placenta to meet them at around 8 to 10 months of gestation. A retrospective analysis of breeding records by Jobert and co-workers (2005), reported the outcome of 30 body pregnancies. Nine of the 30 were present in the cranial body, and 21 were present in the caudal body. The pregnancy loss rate by 45 days was 11 and 62% for cranial and caudal body pregnancies respectively. From Day 45 to term the loss rate was 12 and 40% for cranial and caudal body pregnancies respectively, with abortions occurring between 4 and 9 months of gestation. Therefore there is a greater likelihood that body pregnancies will be carried for the full length of gestation if they are in the cranial uterine body (Jobert et al. 2005).

Fetal abnormalities may also result in abortion, although many are carried to term. Severe developmental anomalies involving the central nervous system or development of body cavities have been reported in aborted fetuses (see Chapter 9).

Tall fescue grass is widely used in the United States in pastures. Much of it is infested with an endophyte (*Neotyphodium,* formerly *Acremonium coenophialum*) that has dopamine agonist activity. Fescue infected with this endophyte actually performs better than noninfected fescue in pastures, hence its widespread distribution. However, when mares graze this fescue during gestation, a number of adverse effects have been reported to occur, namely: abortion, stillbirth, prolonged gestation, dystocia, thickened placenta, agalactia, increased mare mortality, and weak foals. In addition abnormal cyclicity, lower conception rates, and increased EED are also seen in mares grazing on infected fescue. Treatment of pregnant mares is aimed at either removing the mares from the fescue at 300 days of gestation, which will prevent problems at parturition, or treating affected mares with a dopamine antagonist such as domperidone.

Infectious Causes of Abortion

Equine Herpesvirus. Equine herpesvirus (EHV) is the single most important infectious cause of equine abortion. Abortion is primarily caused by EHV-1 and, on occasions, EHV-4. EHV-1 is also capable of causing respiratory disease (most noticeable in foals and yearlings), paralysis, neonatal foal disease, and uveitis/hypopyon. EHV-4 normally causes respiratory disease but occasionally has caused abortion in single mares. Transmission is by the respiratory route. Primary replication of the virus is in the mucosal epithelium, followed by a lymphocyte-associated viremia. The virus persists in the pregnant mare for long periods with the majority of abortions (90%) occurring within 60 days postinfection; however, the range from infection to abortion is 14 to 120 days.

Most mares of breeding age are clinically immune to respiratory disease but not to abortigenic infection. Spread of EHV-1 through a herd most commonly occurs without clinical signs. Immunity is short after natural infection (3–4 months), and individuals may be reinfected repeatedly. Additionally, the virus can persist in the trigeminal ganglia and can recrudesce in latent carriers; thus stressing pregnant mares may trigger abortion.

Abortions may occur from the fifth month of gestation to term but most commonly occur from 8 to 9 months to term. Infection of the fetus occurs by migrating leucocytes and/or infection of umbilical blood vessels, with the primary target organs in the fetus being the respiratory tract and liver. Premonitory signs of abortion are few; thus, typically, there is a sudden abortion without milk or udder development. The fetus is delivered in a fresh condition, often still enclosed in its membranes; rarely, some foals survive for up to 7 days, but they are weak, jaundiced, and have a marked leucopenia.

Diagnosis of abortion is by histopathology of the fetus. Fetal lesions include: serous pleural and peritoneal fluid effusions; jaundice of the mucous and placental membranes; enlargement of the spleen; perirenal oedema; haemorrhages of the entire respiratory tract; petechia of the oral mucosa and conjunctiva; focal hepatic necrosis with intranuclear inclusion bodies in liver and lung; and a necrotic and friable thymus. Specimens for submission to the diagnostic laboratory should include lung, liver, and thymus, both fresh/frozen and in formalin. Virus isolation is possible from lung, liver, and thymus samples that have been submitted in viral transport medium; fluorescent antibody tests can be performed on frozen sections of liver and lung. Diagnosis may also be confirmed by virus isolation or PCR, but unfortunately, there is no test to detect latent carriers. Serology of the mare is of no value in the diagnosis of abortion due to EHV because of the time period that has usually lapsed between maternal infection or viremia and abortion. In addition, the mare may have elevated antibody titres due to previous vaccination. Therefore analysis of the fetus and placental membranes remain the samples of choice.

The focus of control measures varies by country. In most countries control is achieved through vaccination programmes, with both modified live and killed vaccines being available. Controlled studies have showed that both are effective and that both have failures with vaccinated mares aborting. The vaccine is best considered as an aid in the prevention of viral abortion rather than absolute protection. Recommendations are to vaccinate pregnant mares with 3 doses, given at 5, 7, and 9 months of gestation.

Even with vaccination programmes, because immunity is not permanent and recrudescence in carriers may occur, management is important in disease control. Groups of mixed ages and reproductive status are most at risk from virus abortion; thus weanlings, yearlings, and other horses should be kept away from pregnant

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mares. The pregnant mares should be kept in groups by stage of gestation and maintained in relative isolation. All new arrivals should be isolated for 21 days and allowed to settle into their new environment. First foaling mares should be kept away from older mares. If mares are removed from a group, it is best not to reintroduce them as the whole group will have to reestablish their social 'pecking order' and thus be at risk of stress. All abortions and stillbirths should be investigated and the mare isolated pending the results. If the mare aborts in the paddock, the area where the abortion occurred should be cordoned off and the area disinfected. If other mares have had potential contact with aborted materials the whole group/paddock of mares should be considered at risk and thus isolated as a group and managed accordingly. Newly arrived animals, pregnant or otherwise, should never be mixed with pregnant mares on a farm.

Equine Viral Arteritis. Equine viral arteritis (EVA) is a contagious viral disease of horses first identified in 1953. An EVA outbreak was identified for the first time in the UK in 1993 and is subsequently a notifiable disease in the UK. Some outbreaks of the disease are not associated with abortions, whereas other outbreaks are associated with significant pregnancy losses. Outbreaks have been attributed to the use of fresh cooled semen from a carrier stallion. Although naturally occurring abortion from EVA is not common, it does occur sporadically, sometimes in clusters. Some breeds (e.g., Standardbreds) have a high incidence of seropositive individuals, yet disease is almost never seen. Other breeds are quite naïve and clinical signs (i.e., abortion) are more common; there may be some differences in pathogenicity between strains of the virus.

The two important routes of EVA transmission are: venereal from a stallion with infected semen, and aerosol via the respiratory secretions of an acutely infected horse. Close or direct contact is required for aerosol transmission to occur. After an average incubation period of 7 days, EVA is excreted in all bodily secretions, including respiratory secretions and urine for up to 21 days (possibly longer in urine). After infection, mares clear the infection and develop immunity. However, the virus may persist indefinitely in the accessory sex glands in stallions, which then become inapparent carriers and remain so for years, shedding the virus in the semen. It would appear that carrier status is testosterone dependent and exists in 30% to 60% of stallions that are infected (Little et al. 1991); there is no evidence that mares, geldings, or foals that acquire the infection become carriers. Interestingly, marked changes in semen quality in stallions experimentally infected with EVA have been described (Neu et al. 1992). At present, there is no commercially available treatment for a chronically infected shedder stallion. Surgical castration results in rapid cessation of virus shedding; however, this is not an attractive proposition for a breeding stallion. There has been some work to suggest that semen centrifugation using density gradients may reduce the viral load of semen samples from shedding stallions (Morrell et al. 2013); however, no data is available on the ability of processed samples to infect mares. It has, however, been demonstrated experimentally that reducing testosterone concentrations via GnRH vaccination or by means of GnRH antagonist has resulted in conversion to nonshedder status (Burger et al. 2006, Fortier et al. 2002).

Venereal transmission is believed to be the major cause of widespread dissemination of the virus. In breeds that permit the use of artificial insemination, the virus can be transmitted through the use of fresh, chilled, or frozen semen. In a controlled study (Cole et al. 1986), seronegative mares were bred to carrier stallions. The mares were then transported to another location and housed with seronegative mares that were in the seventh to eleventh month of gestation. All mares became seropositive. Clinical signs would have gone unobserved but for the experimental protocol that included twice daily monitoring of body temperature and weighing of feed remaining at the end of the day. A slight febrile response and mild anorexia was detected. Of the exposed mares, 10 of 14 pregnant mares aborted.

Clinical signs of EVA are very variable, and typically, there are no clinical signs other than abortion in mares. The classic clinical signs are: an influenza-like illness with pyrexia for 1 to 5 days; depression; a nasal discharge; conjunctivitis; anorexia; and a focal dermatitis and oedema of the limbs, ventral abdomen, scrotum, prepuce, and periorbital regions. Abortion may occur during or shortly after an acute illness or subclinical infection. The majority of abortions occur 23 to 57 days after exposure, or 6 to 29 days after the onset of fever in the mare. Foals can be born infected and die within 72 hours, appearing normal when born or born ill, then developing respiratory signs or enteritis.

A definitive diagnosis, based on clinical signs alone, is not possible due to their variable nature. Acute EVA can be confirmed by virus isolation from nasopharyngeal swabs, heparinised blood samples, and urine and semen samples. Serological evidence of EVA exposure can be found by taking an initial serum sample as soon as possible after clinical onset, followed by a convalescent sample 10 to 14 days later to detect a rise in the EVA antibody titre. Diagnosis of abortion due to EVA is largely dependent on virus isolation from the placenta or fetal tissues; there are no pathognomonic gross lesions. After abortion the fetal tissues that should be collected for laboratory diagnosis are: lung, liver, placenta, and fetal fluids. If EVA is the cause of the abortion, it is fairly easy to isolate. Mares infected with EVA will usually abort partially autolysed fetuses in contrast to fresh fetuses aborted by mares infected with EHV.

Because virtually all acutely infected horses recover uneventfully after EVA, any treatment is symptomatic. In the UK, the code of Practice considers control measures for EVA and provides excellent guidance in the event of an outbreak. A modified live-virus vaccine is available in North America, whereas a killed vaccine (Artervac, Zoetis) is available in the UK. In the United States, use of the vaccine, although regulated by the various state governments, is becoming more widely practised due to recent outbreaks. There is some reluctance to vaccinate because once vaccinated, a stallion cannot be distinguished from a naturally infected animal by serology alone. Virus isolation from the semen can distinguish seropositive carriers from vaccinates. It must be remembered that certain countries will not accept the importation of seropositive animals or their semen. If an animal is to be vaccinated, a blood sample for serology should be taken before vaccination, followed by a second blood sample taken 10 days after the second vaccination to ensure an adequate serological response to vaccination. Many studs in the UK are requiring confirmation that mares are seronegative to EVA before arrival at stud and exposure to the stallions. Vaccination of stallions has been widely adopted in the UK in Thoroughbred and sportshorse stallions, yet issues with vaccine supply and cost of the vaccine has resulted in less uptake in recent years. The UK is EVA free, yet other areas of mainland Europe are not.

Other measures of control include keeping a distance of about 90 yards between a shedding stallion and other horses or between a mare bred to a shedding stallion and other mares. Mares can be bred to a shedding stallion as long as they are vaccinated at least 21 days before breeding or are kept isolated from other seronegative horses for a month after breeding.

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Leptospirosis. Leptospirosis is a zoonotic disease with worldwide distribution. The predominant serovar affecting horses varies by country and region. *Leptospira interrogans* serogroup *Pomona* serovar *kennewicki* has been reported to be most often implicated in equine abortion in Kentucky. *L. kirschneri* serogroup *Grippotyphosa* serovar *grippotyphosa* has also been reported. The raccoon is the maintenance host for grippotyphosa, but the maintenance host for Pomona serovar *kennewicki* has not been identified.

Favourable conditions are moist, warm environments, and invasion occurs through mucous membranes and soft moist skin. A bactaeremia occurs after an incubation period of 4 to 10 days, with pathogenical serovars localising in the kidneys or genital tract; clinical signs in the mare are often unobserved, with abortions occurring from 6 months to term. Gross lesions of the fetus vary and may include icterus and a swollen liver and/or kidneys, whereas placental lesions are common. Diagnosis is by identification of the organisms by real-time PCR and/or fluorescent antibody test (FAT) and by serology of the mare by microscopical agglutination test (MAT) (Erol et al. 2014); control is achieved by good sanitation, hygiene, and control of wildlife. Some reports indicate potassium penicillin may be useful in pregnant mares with rising titres, whereas human studies indicate that intravenous penicillin in high doses may be a reasonable treatment. A vaccine (Lepto EQ Innovator) is available in the United States to protect against infection by Leptospira interrogans serovar Pomona.

Bacterial/Fungal Abortion. Bacterial/fungal abortion (placentitis) is a leading cause of abortion and neonatal disease in horses. A large number of bacterial species that gain access to the placenta can cause abortion in the mare, with the ascending pathway via the cervix being the primary route of infection. Rarely, bacteria may be in the uterus at the time of conception or arrive haematogenously afterwards. Bacteria that spread rapidly through the allantochorion often infect the fetus, causing acute bacterial septicaemia. More chronic ascending infections are often localised around the cervical star and cause a focal or local placentitis (Fig. 31.14), which often leads to placental insufficiency with abortion of a growth-retarded fetus or the birth of a dysmature foal. The placenta is often thickened and covered with exudates, and the fetus is septicaemic. Bacteria that cause placentitis are similar to the organisms that cause endometritis (see later in this chapter); they are often opportunist pathogens that can be isolated from the caudal genital tract of normal mares. Normally, there are premonitory signs of an impending abortion, such as premature mammary gland enlargement, premature lactation, and relaxation of the vulva.

• **Fig. 31.14** Ascending placentitis; note the discoloured, thickened area at the cervical pole.

Early diagnosis is critical to be able to begin effective treatment that can result in the survival of a healthy foal. The ability to diagnose placentitis early, before clinical signs of premature lactation or vaginal discharge are observed, will improve the chances of successful treatment. Although it cannot be recommended to examine all mares routinely at intervals for placental abnormalities, mares with a history of problems or at high risk may be periodically examined for signs of placental disease.

Reef and co-workers (Reef et al. 1996) have described the assessment of fetal well-being in utero using a transcutaneous approach. The uterus is examined at multiple sites, attempting to cover all four quadrants. Fetal heart rate, the diameter of the fetal aorta, fetal activity, maximum depth of fetal fluids, utero placental contact, and utero placental thickness have been found to be related to the pregnancy outcome. Significant utero placental thickening is consistent with placentitis, whereas anechoic spaces between the uterus and placenta suggest placental separation. Although observed abnormalities are good indicators of placental disease, the lack of abnormal signs on transcutaneous examination does not mean that placental disease does not exist; focal areas of placentitis may go undetected.

Most cases of placentitis stem from the spread of bacteria through the cervix, infecting the chorioallantois in the region of the cervical star, and spreading from there. Renaudin and others (1999) have examined using transrectal ultrasonography the utero placental unit near the cervical os, near the area where most cases of placentitis would be expected to originate. Measurements of the combined thickness of the uterus and placenta (CTUP) at the ventral portion of the uterus, rostral to the internal cervical os, are obtained. The normal thickness of the utero placental unit is dependent on gestational age (8 mm at 270–300 days gestation, 10 mm at 300–330 days, 12 mm > 330 days). Normal values for the CTUP have been established in a variety of breeds; the authors generally use those determined in Quarter Horses (Renaudin et. al. 1997) as a guide. Multiple measurements should be taken and the mean value obtained because a single value may be misleading, especially if the image is taken at an oblique angle. Placentitis has been demonstrated to cause a marked increase in CTUP, and thicknesses higher than the normal ranges have been associated with pending abortion and placental pathology. Although an elevated CTUP may suggest pathological changes, mild increases may require confirmation by the presence of other clinical signs. Conversely, not all mares suffering from an ascending placentitis show ultrasonographically detectable placental thickening.

Other features of ascending placentitis are placental separation with accumulation of purulent material/fluid between the uterus and placenta. Measurements in these cases are overruled by the presence of separation, which is strongly indicative of an ascending placental infection (Fig. 31.15).

Speculum examination of mares with a purulent discharge usually reveals an exudate originating from the cervix; this will also enable swabbing of the cervix in order to determine the aetiological agent(s) and their sensitivity pattern. In addition, progestagen and total oestrogen concentrations can be measured to assess the health of the fetoplacental unit. Mammary electrolytes are unreliable for predicting readiness for birth in cases of placentitis. Serial hormone measurements have been suggested as a means of diagnosing pathological changes, with mares with acute placentitis often demonstrating a rapid decline in plasma progestagen concentrations, whereas mares that develop chronic placentitis often have increased plasma

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• **Fig. 31.15** Ultrasonographic image of the combined uterus and placenta, near the internal cervical os. The placenta and uterus are separated by a hyperechoic exudate.

progestin concentrations. Ball and co-workers (2013b) demonstrated that concentrations of oestradiol-17β sulphate declined precipitously after experimentally induced bacterial placentitis, suggesting that maternal oestrogen concentrations may be useful as an early marker of a placental insult. Recent work by Canisso and co-workers (2014) demonstrated that concentrations of the acute phase proteins serum amyloid A (SAA) and haptoglobin (Hp) rapidly increased subsequent to experimental induction of placentitis and remained elevated until abortion. Neither fibrinogen nor white blood cell concentrations appeared to be useful markers for placentitis in this study. Parturition did not trigger an increase in either SAA or Hp in normal foaling mares. SAA, however, is also a very nonspecific indicator as many other acute inflammatory conditions (i.e., vaccination) may result in an elevation of SAA.

If placentitis is diagnosed via clinical signs or detection of an increased CTUP, treatment should be initiated immediately to ensure the best case outcome; parturition should not be induced. The longer the fetus is carried in utero, the better chance it has to reach a level of maturity that will allow it to survive after birth. If a vaginal discharge is present, culture and sensitivity may be beneficial in choosing an appropriate antibiotic. The treatment strategy is aimed at controlling the infection, reducing the associated inflammation and promoting myometrial quiescence. Antibiotic choice should be determined by culture and sensitivity results. If these are not available, broad spectrum antibiosis should be instigated. Trimethoprim sulfadiazine (30 mg/kg, bid) or a combination of potassium penicillin G (22,000 IU/kg, qid) and gentamicin (6.6 mg/kg, sid) have been demonstrated to penetrate placental and fetal tissues and are therefore the antibiotics of choice. Despite ceftiofur sodium being found to penetrate endometrial tissues, it has not been found to cross to the fetal membranes, and thus it is not recommended for cases of placentitis (Macpherson et al. 2014).

Many treatment regimens have been suggested – many extrapolated from other species – and have been largely anecdotal. Progestins are advocated to promote uterine quiescence in mares with high risk pregnancies and have been demonstrated to prevent prostaglandin induced myometrial activity; they are effective in preventing prostaglandin induced abortion. Tocolytics, such as isoxsuprine and clenbuterol, have been shown to cause short-term uterine relaxation; however, they may not be of practical use in cases of placentitis. Nonsteroidal antiinflammatories, such as flunixin meglumine and phenylbutazone, are obvious choices to reduce inflammation and prevent prostaglandin-mediated abortion. Other agents have been investigated, including pentoxifylline, which is used clinically in humans as a peripheral vasodilator and has been demonstrated to down regulate proinflammatory cytokines and reduce the mortality rate in premature human infants with sepsis. Recent data from Bailey and co-workers (2012), however, suggests that short term therapy with pentoxifylline does not affect uterine blood flow in the mare. Acetylsalicylic acid (aspirin) has been demonstrated to reduce the incidence of preeclampsia in women with abnormal placentation and has been demonstrated to improve uterine blood flow in the mare.

Recent research with experimental models of placentitis would suggest that aggressive antibiotic therapy with trimethoprim sulfadiazine alone can substantially improve pregnancy outcome (Christiansen et al. 2010). The addition of aspirin (50 mg/kg, PO, bid) resulted in a marginal increase in the percentage of viable foals born in this study. A combination of trimethoprim sulfadiazine (30 mg/kg, PO, bid), altrenogest (0.088 mg/kg, PO, sid), and pentoxyfylline (8.5 mg/kg, PO, bid), however, would appear to be the recommended therapy at this time (Bailey et al. 2007). A specific cause of placentitis, found primarily in Kentucky and the surrounding area, has been identified as a nocardioform organism, *Crossiella equi.* This disease is characterised by an area of placentitis with a tan mucoid exudate located at the base of a uterine horn near the body of the placenta.

Aspergillus spp. is the most common cause of mycotic placentitis and abortion in the mare. Other fungi such as *Mucor sp.* have been reported; much less common is placentitis due to *Candida spp.* infection. Fungal disease can have a similar pathogenesis to bacterial abortion with inflammation of the chorion beginning at the cervical pole. Infection may also originate from the respiratory tract, through the circulatory system, to the uterus and placenta. Typical gross lesions of the placenta include necrotic areas that are difficult to distinguish from bacterial lesions. The chorionic surface may be dry, thick, and leathery. There may be lesions on the fetus, especially the skin. Fungal elements are easily demonstrable in smears and cultures if they are responsible for the abortion. Before term, these cases are very difficult to manage due to the difficulty in administering antifungal medications, and cases often result in abortion.

Endometritis

Endometritis, an acute or chronic inflammatory process involving the endometrium, has been recognised for many years as a major cause of infertility in brood mares. This subfertility is due to a hostile environment for the developing conceptus, and in some cases, the endometritis causes early regression of the CL through inflammation and endogenous prostaglandin release. Endometritis frequently occurs as a result of microbial infection but can also be due to noninfectious causes.

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The underlying aetiology of the specific cause of endometritis determines the type of treatment to be used, and the following classification system for equine endometritis is useful:

- Venereal infection
- Chronic infectious endometritis
- Persistent breeding-induced endometritis (PBIE) caused by delayed uterine clearance (DUC)

In the past it was generally assumed that the uterine lumen of the normal fertile mare is bacteriologically sterile or may have a temporary, nonresident microflora. This is despite the fact that the mare's reproductive tract is often contaminated with bacteria from the act of coitus, foaling, and veterinary procedures. In addition, mares with a defective vulvar conformation can also aspirate air, bacteria, and debris into the vagina that can develop into endometritis.

The bacterial species that cause bacterial endometritis are numerous and can be classified as follows: (1) contaminants and commensals; (2) opportunists, causing an acute endometritis; and (3) venereally transmitted. Normally, the vestibular and clitoral area has a harmless and constantly fluctuating bacterial population. In association with benign saprophytic organisms, opportunistic organisms such as *Streptococcus zooepidemicus, E. coli,* and *Staphyloccocus spp.* can be found. The stallion's penis is colonised by similar organisms. *S. zooepidemicus* is the most commonly isolated bacterial species from acute endometritis, particularly in the initial stages. *E. coli* is the next most common isolate. The uterus responds to these bacteria with a rapid influx of neutrophils (Pycock & Allen 1990). Normally, these neutrophils phagocytose and kill the bacteria rapidly (<24 hours). The inflammatory byproducts are then mechanically evacuated, and the endometritis resolves itself except when the mare suffers from pneumovagina or is a 'susceptible' mare. Susceptible mares have a delay in uterine clearance and fail to resolve the inevitable postbreeding endometritis, with inflammatory byproducts accumulating as intrauterine fluid. Such mares have a reduced pregnancy rate due to a hostile environment for the early developing conceptus.

Venereal Endometritis

In addition to opportunist pathogens, some bacteria may be venereally transmitted – e.g., *Taylorella equigenitalis* (contagious equine metritis organism, CEM), *Klebsiella pneumoniae* (capsular types 1, 2 and 5), and *Pseudomonas aeruginosa* (some strains) – by asymptomatic carriers of either sex. Mating or gynaecological examination may result in their transfer into the uterus. Stallions may harbour the organisms over the entire surface of the penis and in the distal urethra. Control is by routine screening of swabs taken before mating by laboratories experienced in the isolation and identification of these specific organisms. Anaerobic bacteria have been isolated from the mare's uterus, with *Bacteroides fragilis* the most frequent (Ricketts & Mackintosh 1987). Further work is needed to assess the importance of anaerobes in endometritis.

Diagnosis. Before the breeding season, swabs should be taken from the clitoral fossa, clitoral sinuses (only the medial sinus may be obvious), and the vestibule. The perineal area of the mare should not be cleaned except for the removal of gross contamination of the vulva with faeces using a dry paper towel. A protective disposable glove should be worn by the veterinary surgeon on the hand used to evert the ventral commissure of the vulva and expose the clitoris. The swabs should be placed in transport medium, clearly labelled with the mare's name, and sent to an approved laboratory. It is important to penetrate the clitoral sinus, and therefore a large swab tip should not be used. Swabs are cultured aerobically on blood and MacConkey's agar to screen for the presence of *K. pneumoniae* and *P. aeruginosa.* Microaerophilic culture on chocolate blood agar (with and without streptomycin) must also be done for the detection of CEM if not in a CEM-free country. In addition, in stallions, multiple swabs must be taken from the urethral orifice, ideally before and after ejaculation, with the former sample being of preejaculatory fluid, urethral fossa, and/or sinus, penile sheath, and semen. In the UK, this screening has been successful in virtually eradicating CEM and vastly reducing the incidence of venereal disease.

Treatment. Any mare that is suspected of having a venereal infection must not be bred. In the case of clitoral or vestibular infections, topical treatment is used. This involves thorough cleaning with chlorhexidine surgical scrub followed by the application of 0.2% nitrofurazone ointment for *T. equigenitalis,* 0.3% gentamicin cream for *K. Pneumoniae,* or silver nitrate and gentamicin cream for *P. aeruginosa.* These pathogens, particularly *P. aeruginosa,* are difficult to eliminate from the clitoris; hence clitoral sinusectomy or clitorectomy may have to be used in refractory cases. A broth culture containing a mixture of growing organisms prepared from the normal clitoral flora can suppress venereal pathogens in some cases. Evidence for the successful elimination of infection is based on three negative sets of clitoral and endometrial swabs, taken at weekly intervals.

Chronic Infectious Endometritis

Chronic infectious endometritis is found most frequently in older mares that have had several foals. Such mares have compromised uterine defence mechanisms that allow the normal vestibular and vaginal flora to colonise the uterus, thus inducing a chronic endometritis. A diagnosis of endometritis can be made by collection of samples during oestrus for bacteriological culture and cytological examination. The veterinarian must verify that the mare is not pregnant before passing a swab through the cervix. The ideal technique should ensure that the samples are obtained from the uterine lumen only. It is important to ensure that the method of swabbing does not introduce bacteria into a previously normal uterus. A guarded swab is passed into the uterine lumen, and the swab tip is exposed only when it is in the uterine lumen. A swab for cytological examination should also be taken. A flaw with this technique is that the swab only samples a small area of the uterus, usually in the more caudal regions of the uterine horn or body. It has been suggested that the type of bacteria influences the exudate produced by the endometrium, which in turn affects the ability to obtain a representative cytology sample by swabbing (LeBlanc et al. 2007). A low volume lavage (LeBlanc et al. 2007) is a preferable method in many cases, especially in cases of negative culture results even when associated with infertility. To utilise this technique, a small volume (100 mL) of saline or lactated Ringers is introduced into the uterus in an aseptic manner with a large bore catheter. The lavage fluid is then massaged in the uterus per rectum in order to cover a greater percentage of the endometrial surface and then retrieved into a sterile vessel such as a 50 mL centrifuge tube. If the retrieved fluid is not clear it should be centrifuged, and the pellet used for culture and cytological examination. More recently, a double-guarded low volume lavage technique using 250 mL of fluid has been described (Christoffersen et al. 2015). Another option for the collection of cytology samples is to use a cytobrush. The low volume lavage and cytobrush are both superior to the use of the cotton swab (Overbeck et al. 2011, Cocchia et al. 2012), yet due to the fact that they are more sensitive, more studies

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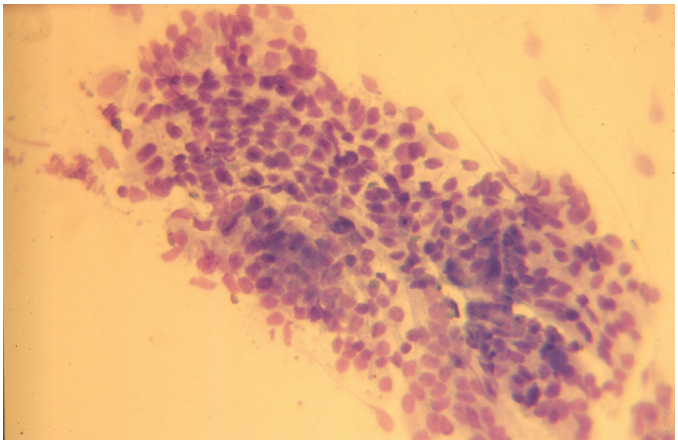
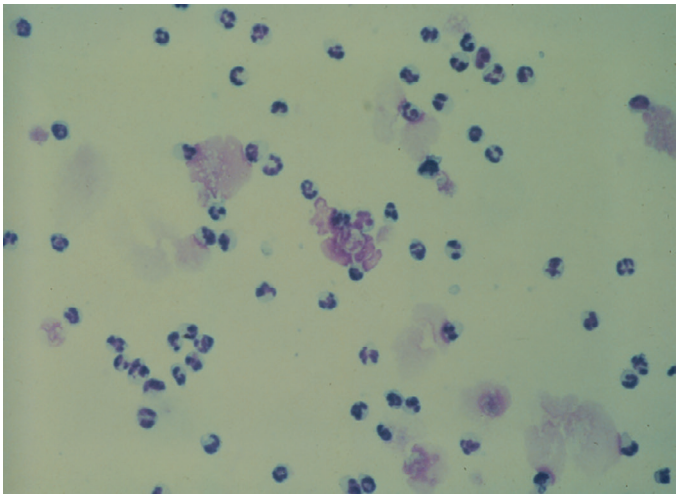
are needed to establish criteria for interpretation of inflammation (Cocchia et al. 2012).

Although *Strep. zooepidemicus* typically incites a strong inflammatory response, resulting in the identification of intraluminal fluid on transrectal ultrasound examination and a positive cytology (and culture), it has been shown that other pathogens such as *E. coli* may not incite the inflammatory response to the same degree, and cytology (and culture) may be negative (LeBlanc et al. 2007). Swabs for culture should be plated on blood and MacConkey's agar and incubated at 37°C for 48 hours. Chromogenic agar plates are used by some practitioners in addition to blood and MacConkey's to aid in the identification of the bacteria isolated (Beehan & McKinnon 2009); cultures should be examined at 24 and 48 hours. An air-dried smear for cytology is made by gently rolling the other swab on a microscope slide. The smear is then stained with a rapid modified Wright's stain such as Diff-Kwik (American Hospital Supplies); the stained smear should be examined for the presence of inflammatory and endometrial cells (Fig. 31.16), the latter confirming that a satisfactory sample was obtained. Uterine cytology provides critical information that assists in interpretation of culture results, and diagnosis of endometritis is based on the presence of significant numbers of neutrophils in the smear (Fig. 31.17). Mares that have more than 5 neutrophils/high power field (X400) present in a cytology smear should be considered to have active endometritis.



• **Fig. 31.16** 'Negative' endometrial cytology: numerous endometrial cells, no neutrophils.



• **Fig. 31.17** 'Positive' endometrial cytology: numerous neutrophils.

If certain pathogens (e.g., *Strep. zooepidemicus*) are present in the uterus, the mare's immune system will mount a response, and neutrophils will usually be observed in a cytology sample. If the cytology sample is 'negative', it means that there are sufficient endometrial cells present to make a diagnosis, and if there are no or rare neutrophils, then bacteria that result from the culture taken may be contaminants inadvertently picked up during the procedure. However, in some cases a positive culture with a negative cytology warrants treatment (LeBlanc et al. 2007).

In Table 31.1, the possible outcomes are outlined. First, if the cytology is 'positive', then the culture should be submitted to the laboratory to identify the pathogen and determine its antibiotic sensitivity. If a pathogen is not identified (i.e., 'no growth'), yet the cytology is positive, various explanations exist. The mare may be recovering from a previous infection, and although residual inflammation is evident, the pathogen has been eliminated. Alternatively, some other cause of noninfectious inflammation may be present. Then again, the mare may have a uterine infection, but a sample handling or laboratory error killed the pathogen, resulting in a negative culture result. It has also been suggested that anaerobic bacteria will result in a positive cytology but fail to yield results with standard aerobic culture techniques (Ricketts & Mackintosh 1987). In the case of a positive cytology and negative culture, a decision must be made, based on history and other findings, whether to repeat the culture and cytology procedure or treat the mare. If treatment is considered, modalities such as uterine lavage rather than antibiotic infusion may be preferable.

In some cases, an endometrial biopsy may be a useful diagnostic aid. It has been suggested that culture and cytology (via histopathology) from an endometrial biopsy may be the gold standard as it is more sensitive with a greater positive predictive value compared with cotton swabs (Nielsen 2005). This topic is covered in more detail later in this chapter, under "Uterus". For detailed reviews of the clinical application and pathological findings, readers should consult Kenney and Doig (1986).

Ultrasonography is indispensable in the diagnosis of endometritis. Since the first description of the identification of the collection of small volumes of intrauterine fluid using ultrasound, which could not be palpated per rectum (Ginther & Pierson 1984), general awareness of the frequency of this abnormality has increased. Endometrial secretions and the formation of the small volume of free fluid may be associated with the same mechanism that causes normal oestral oedema (Fig. 31.18). In many cases, the uterine luminal fluid that accumulates before mating is sterile and contains no neutrophils (Pycock & Newcombe 1996a). However, free fluid in the lumen is a warning sign that the mare may have problems

TABLE 31.1 Possible outcomes of endometrial culture and cytology examination

	Positive Cytology	Negative Cytology
Positive culture	Pathogen identified Treat based on results	False positive (Contaminant)? Don't treat? Treat? Recheck?
Negative culture	Reculture? Treat?	Do not treat

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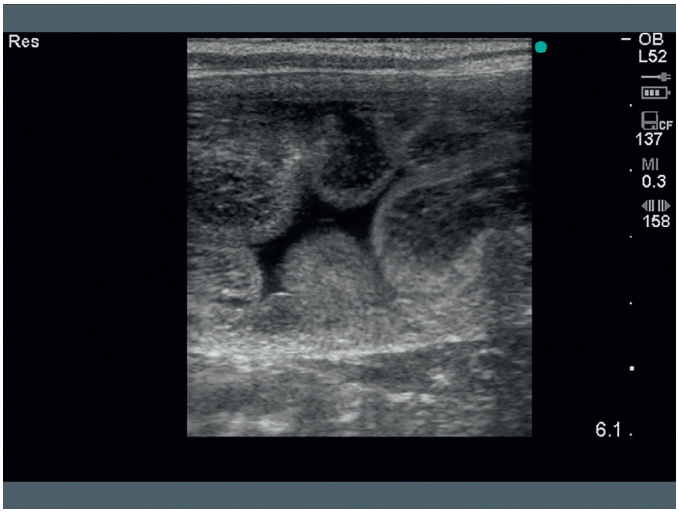
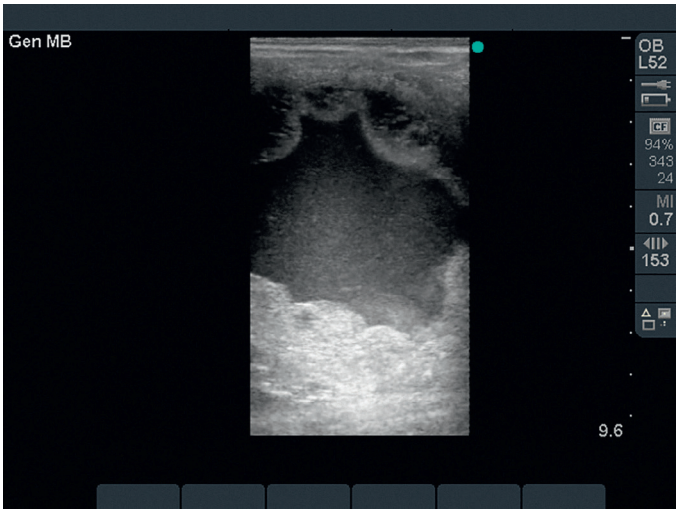


• **Fig. 31.18** Uterine oedema and a small amount of fluid in the lumen of the uterine horn, typical of early oestrus to midoestrus.



• **Fig. 31.19** Excessive uterine fluid in the lumen of the uterine body, indicative of severe endometritis.

with delayed uterine clearance (see persistent breeding-induced endometritis (PBIE) later in this chapter). The amount of fluid that should be considered significant is not clear. The significance depends to some extent on when during oestrus the fluid is observed; fluid detected early in oestrus may have disappeared when the mare is further advanced in oestrus and the cervix relaxes more. Small volumes of intrauterine fluid during oestrus do not affect pregnancy rates, in contrast to mares with larger (> 2 cm depth) collections of fluid (Pycock & Newcombe 1996a, Brinsko et al. 2003). In mares that are susceptible to endometritis, there is an accumulation of more fluid than in nonsusceptible mares.

Generally, if there is more than 1 cm of fluid during oestrus, some attempt should be made to remove this before breedin g using oxytocin treatment. If the volume is more than 2 cm (Fig. 31.19), the fluid should be investigated for the presence of inflammatory cells and bacteria. Intrauterine fluid during dioestrus is indicative of inflammation and associated with subfertility due to early embryonic death and a shortened luteal phase (Newcombe 1997).

Transrectal ultrasonography provides a rapid, noninvasive method of assessment of the uterus. In a study involving ultrasonographic examination, cytological, and bacteriological sampling of the uterus in 380 brood mares before mating (Pycock & Newcombe 1996a), it was concluded that if no free fluid is detected during oestrus, then acute endometritis as detected in cytology is absent in 99% of cases.

The most favoured approach to treatment has been the infusion of various antibiotics into the uterine lumen during oestrus. The intrauterine route is usually preferable to systemic therapy, as most acute endometritis cases are localised. Systemic treatment alone, or in combination with local application, is suitable in a few circumstances. Ideally, the choice of antibiotic for local treatment should be based on *in vitro* antibiotic sensitivity tests. However, in many cases this is not possible and a broad spectrum antibiotic or combination of antibiotics should be used that is effective against the mixed aerobic and anaerobic infections that commonly occur. Although 35 to 40 mL is a sufficient volume to cover the entire endometrial surface in most mares, a larger volume (up to 100 mL) may be better in older, pluriparous mares to ensure distribution throughout the uterus. Larger volumes will flow back out through the cervix and be lost. The number of treatments required depends on individual circumstances and the inciting pathogen, but a single treatment may be effective or daily infusions for 3 to 5 days during oestrus are effective in most cases, especially if combined with oxytocin. The success of treatment can be monitored using ultrasonography to identify the presence of intrauterine fluid. Repeated endometrial swab/smear or biopsy examinations may be used to monitor the response to therapy; however, every time the cervix is breached there is the risk of introducing more bacteria.

Immunomodulators have been used as treatment adjuncts for endometritis. Available products use a mycobacterium cell wall extract or propionobacter preparations. Although it is not yet clear the exact mechanism by which fertility is improved, there is some evidence that, in some mares, treatment is helpful (Rogan et al. 2007).

In addition to the antibiotic therapy, repeated treatment with PGF increases the frequency of the follicular phases, thus allowing intrauterine therapy to be used more readily. It also reduces the duration of the luteal phase in which progesterone increases the susceptibility to infection. Predisposing causes to the chronic endometritis, such as defective vulvar or cervical conformation, should also be treated.

Recurrent infections with the same bacterial or fungal agent are frustrating to deal with, and it is not uncommon for the clinician to treat one infection only to culture a different one at the next cycle. Of particular interest are agents which colonise in an extracellular matrix known as a biofilm. Biofilm formation is a highly complex process involving the switch from a free floating existence to a sessile (attached) mode of growth. The latter enables the biofilm community to be protected from adverse environmental conditions, such as desiccation, as well as antimicrobial agents. Approximately 80% of the Gram-negative bacteria, e.g., *E. coli, Klebsiella pneumoniae, Pseudomonas aeruginosa,* cultured from the equine uterus are able to produce biofilm *in vitro* (Ferris et al. 2014), and although *Candida* spp. are usually referred to as fluid living, they are known biofilm producers. Beehan and co-workers (2015) demonstrated that approximately 30% of *E. coli* cultured from the reproductive tracts of mares had biofilm-forming potential and subsequently demonstrated for clarity by scanning electron microscopy that these isolates were capable of forming a biofilm colony. In addition, Ferris and co-workers (2016) demonstrated that *P. aeruginosa* was capable of producing biofilm-like material *in vivo*.

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Solutions of acetylcysteine (ACE), hydrogen peroxide, ethylenediaminetetraacetic acid (EDTA)-Tris, and dimethyl sulfoxide (DMSO) have been used clinically to treat uterine infections suspected to be complicated by biofilm presence, but only limited evidence from controlled studies is available. *In vitro* studies (Ferris et al. 2014, Ferris et al. 2016, Loncar et al. 2017) have assessed the effectiveness of ACE, EDTA-Tris, gallium nitrate, hydrogen peroxide, DMSO, and antimicrobial peptide mimic (APM; Ceragyn) as antibiofilm treatments. They have cumulatively demonstrated that no one treatment consistently prevents biofilm biomass in all bacterial isolates. These bacterial species-specific results stress the importance of acquiring organism identification when developing a treatment plan for bacterial endometritis because not all treatments are effective against every bacterial species (Loncar et al. 2017). It is the author's preference to use a 10% to 20% solution of DMSO in 1 L lactated Ringer's as an antibiofilm agent; it also seems to be particularly useful in mares with a significant amount of mucus (Causey et al. 2000). It is the author's (JRC) preference to use a 10% to 20% solution of DMSO in lactated Ringer's as an antibiofilm agent; it also seems to be particularly useful in mares with a significant amount of mucus.

Recent work has indicated that *Streptococcus equi* subspecies *zooepidemicus* may reside deep in the endometrium in a dormant state (Petersen et al. 2009). Petersen and co-workers (2015) suggested that some strains of *S. zooepidemicus* were able to form 'persister cells' and acquired a tolerance to penicillin by means of their dormancy. They subsequently demonstrated, in a population of subfertile mares, that it was possible to activate the dormant persister cells by means of an infusion of a proprietary product (bActivate).

Fungal Infections. Mycotic endometritis is not as common as that of bacteriological origin, but recognition of a fungus as the causal agent is important because commonly used intrauterine antibiotic therapy is ineffective. In cases of fungal endometritis, mares may have a history of normal or abnormal oestrous cycles, they may be anoestrus or barren, they may have had a recent abortion or fetal membrane retention, or there may be a history of repeated intrauterine antibiotic therapy. Yeasts more frequently cause endometritis than moulds, with *Candida albicans* being the most common isolate.

The diagnosis is based upon the presence of fungal elements and inflammatory cells in endometrial smears. Yeasts can often be identified after staining with Diff-Kwik using a magnification of X400. Fungal elements are more readily identified in endometrial biopsies after staining with Gomori's methenamine silver or periodic acid–Schiff (PAS). Successful culture of endometrial smears for fungi can be difficult because the organisms may be present in low numbers, and furthermore, they require a long incubation period.

Fungal infections are very difficult to treat, particularly if they are chronic or deep-seated; uterine biopsy may be useful to determine how deep the infection is within the endometrium. Ideally fungal culture and sensitivity to antimycotics would be performed in all cases; however, such diagnostic facilities are not always accessible or economic. A study by Beltaire and co-workers (2012) reported on 102 fungal isolates and demonstrated, within their referral population of samples in North America, a 95% to 100% susceptibility of isolates to the polyenes and a 47% to 81% susceptibility of isolates to the azoles. Uterine lavage with 2 to 3 litres of saline or dilute povidone-iodine (1%–2% solution daily for 5 days), followed by intrauterine antimycotic preparations such as nystatin (5 g or 500,000 units daily for 5 days) or clotrimazole (400–600 mg daily for 6 days) have been used with limited success. Lufeneron, an insect growth inhibitor, has been reportedly used with some success (Hess et al. 2002); however, these results have not been repeated in practice. Uterine irrigation with acetic acid or vinegar has also reportedly been successful in conjunction with antifungal agents, perhaps enhancing efficacy of therapy by altering the uterine pH. However, treatment failure rates are high, and may be in the region of 40% to 70%. Some clinicians have suggested that the addition of a systemic antifungal agent, such as fluconazole for 2 to 3 weeks, reduces treatment failure rates. As discussed previously, if fungal biofilms are present, other 'antibiofilm' therapies may be indicated in the treatment of fungal endometritis.

The prognosis for the subsequent fertility of mares with mycotic endometritis is poor. If there is no success in eliminating the yeast or fungal infection after three attempts, the owner must be advised of the unlikely chance of success. It is suggested that a normal healthy uterus can eliminate mycotic infection; this means that, even if the mycotic infection is successfully treated, the mare must be treated as a susceptible mare.

Persistent Breeding-Induced Endometritis

Persistent breeding-induced endometritis (PBIE) associated with delayed uterine clearance has been shown to be a significant cause of reproductive failure in mares. At coitus, semen, along with microorganisms and debris, is deposited directly into the mare's uterus. Spermatozoa induce an inflammatory response in the uterine lumen (Kotilainen et al. 1994), (Troedsson et al. 2001), which is the same for both live and dead spermatozoa (Katila 2001). In most mares, this transient endometritis resolves spontaneously within 24 to 72 hours so that the environment of the uterine lumen is compatible with the establishment of pregnancy. It is important not to regard this endometritis as a pathological condition but rather a physiological reaction that aids in removing excess sperm and debris from the uterus before the embryo descends from the uterine tube into the uterine lumen 5.5 days after fertilisation.

Classically mares are described as being susceptible or resistant to PBIE, the classification of which is dependent on the mare's response to breeding. If the physiological endometritis persists, in addition to being incompatible with embryonic survival, the premature release of PGF may result in luteolysis and an early return to oestrus. These mares are referred to as susceptible, and they develop a persistent endometritis (Allen & Pycock 1988). Resistant mares can eliminate induced infection without treatment, whereas susceptible mares cannot (Hughes & Loy 1969). Older mares with chronic inflammatory changes within the endometrium (Troedsson et al. 1993) or intrauterine fluid in dioestrus (Troedsson 1995) are likely to be susceptible, although it has been reported that some maiden mares may also be 'susceptible' (Malschitzky et al. 2006). Susceptibility to endometritis is not an absolute state because failure of uterine defence mechanisms need only slow the process of eliminating infection. There is a wide range of susceptibility to endometritis, and mares cannot be neatly categorised into 'resistant' or 'susceptible' (Pycock et al. 1997). Studies on immunoglobulins, opsonins, and the functional ability of neutrophils in the uterus of susceptible mares have not confirmed the presence of an impaired immune response (see the review by Allen & Pycock 1989).

Reduced physical drainage may contribute to an increased susceptibility to uterine infection (Evans et al. 1986). The physical ability of the uterus to eliminate bacteria, inflammatory debris, and fluid is now known to be the critical factor in uterine defence. Experimentally, a failure to clear an intrauterine bacterial challenge (Troedsson et al. 1993b) or the ability to clear intrauterine

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radiocolloid within 2 hours defines susceptibility (Troedsson et al. 1991, LeBlanc et al. 1994). Delayed uterine clearance is primarily due to impaired uterine contractility (Troedsson et al. 1993b) and impaired lymphatic drainage (LeBlanc et al. 1995); however, this is compounded by anatomical deficiencies that lead to a more pendulous uterus or a breakdown in the vulvovestibular or cervical seals. Conversely, a cervix that fails to relax during oestrus, such as that encountered in older maiden mares, will likely inhibit fluid clearance. Retention of fluid, inflammatory cells, and inflammatory by-products, with or without bacterial colonisation, is understandably not conducive to the establishment of pregnancy.

The reason susceptible mares have this defective contractility is not known. Regulation of muscle contraction by the system may be impaired (Liu et al. 1997).

Identification of susceptible mares can be difficult as there may be only subtle changes in the uterine environment not readily detected by current diagnostic procedures. Often in a clinical setting, diagnosis is made postbreeding, when the optimum window for therapeutic intervention may have passed. Many mares show no signs of inflammation before mating, but the inevitable endometritis that follows mating persists. Although the response to bacterial or semen challenge has been used in research studies, history is perhaps the most useful indicator of a susceptible mare in practice. Transrectal ultrasonography to detect uterine luminal fluid after breeding has proved useful in identifying mares with a clearance problem and is the most useful technique available in practice. The presence of free intraluminal fluid before breeding strongly suggests susceptibility to persistent endometritis (Pycock & Newcombe 1996a, Brinsko et al. 2003).

Various treatments are available to enhance uterine clearance and reduce the effects of persistent mating-induced endometritis, including uterine ecbolics, uterine lavage, and intrauterine antibiotics. The success of such treatments can be influenced by a number of factors, such as time of treatment in relation to ovulation and mating and other medications that may be administered concurrently.

Uterine activity is greater during oestrus when the concentration of progesterone is low than when progesterone concentrations are elevated (Cross & Ginther 1987). Myometrial activity and the increase in intrauterine pressure after exogenous oxytocin are inversely related to the concentration of progesterone (Gutjahr et al. 2000). Moreover, reduced uterine clearance is observed under the influence of progesterone (Evans et al. 1986, Evans et al. 1987). The inhibition of myometrial activity by elevated progesterone may necessitate modifications to treatment protocols in the postovulatory period. It is preferable to treat mares with uterine clearance problems before ovulation rather than after. When comparing the effects of 10 or 25 IU oxytocin on intrauterine pressure, although both were effective preovulation, only the high dose increased pressure postovulation (Gutjahr et al. 2000). Consequently, when using oxytocin after ovulation, the dose should be increased to enhance uterine clearance. Assuming good quality semen is used, mating a problem mare earlier, for example 48 hours rather than 24 hours before ovulation, may be beneficial, thus allowing a longer period for treatment to improve uterine clearance before progesterone concentrations begin to rise.

Oxytocin has been shown to be more effective than PGF or its analogues in the short term for enhancing uterine clearance (LeBlanc 1997). Yet, because of the relatively short half-life of oxytocin (Paccamonti et al. 1999), drugs with a longer course of action have been sought. Although the increase in uterine activity after administration of PGF analogues, e.g., cloprostenol, does not begin as early as after oxytocin, the increase in activity persists much longer.

An important factor to consider when using prostaglandin analogues for treatment of delayed uterine clearance is the time of administration in relation to ovulation. Although it is widely accepted that the equine CL is refractory to the luteolytic effect of prostaglandin until five or 6 days postovulation, this is not absolute. In early reports examining the timing of prostaglandin administration, two of five mares treated with PGF 3 days after ovulation responded by returning to oestrus within 2 days (Oxender et al. 1975). Furthermore, more recent work has shown repeated small doses of prostaglandin to be more effective than a single larger dose in inducing luteolysis and a return to oestrus (Irvine et al. 2002).

An additional concern is evidence that even when luteolysis does not occur, luteal function may be affected, at least temporarily, by the use of cloprostenol, a prostaglandin analogue, in the early postovulatory period. Numerous studies have shown that mares treated in the postovulatory period had lower concentrations of progesterone during the ensuing early to middioestrus; however, luteal function seems to be able to recover by the end of dioestrus (Mocklin et al. 2006, Nie et al. 2003, Troedsson et al. 2001). The eventual effect on the establishment of pregnancy is not clear but warrants consideration when prescribing treatment. Further work is needed to clarify the effect of prostaglandins or analogues administered in the early postovulatory period on the establishment of pregnancy. Nevertheless, reports to date indicate that the time of treatment in relation to ovulation as well as the dose and number of treatments should be considered when using prostaglandin or analogues for improving uterine clearance. Although either oxytocin or prostaglandin analogues are effective and safe in the preovulatory period, prostaglandin analogues are best avoided after ovulation.

A long-acting analogue of oxytocin, carbetocin (Reprocine, Vetoquinol), is available and may be useful in situations in which a more prolonged uterine contraction is desired. It has been shown to be safe and effective at inducing uterine clearance. Two intramuscular injections of 0.14 mg carbetocin are given 12 and 24 hours after breeding in mares with marked uterine oedema or free fluid before breeding, or in mares with more than 2 cm uterine fluid 12 hours after breeding.

Another factor to consider in the pharmacological treatment of delayed uterine clearance is the concurrent use of other pharmaceuticals, such as tranquilisers or nonsteroidal antiinflammatory agents. The uterotonic effect seen after oxytocin administration is due to both a direct effect on the myometrium and also indirectly via a release of prostaglandin (Paccamonti et al. 1999). Nonsteroidal antiinflammatory drugs, such as phenylbutazone, can decrease uterine clearance by interfering with endogenous prostaglandin release. Phenylbutazone was found to inhibit uterine clearance, but when mares were pretreated with phenylbutazone before being treated with oxytocin, clearance of radiocolloid from the uterus was restored to that which occurred in control mares treated with only oxytocin (Cadario et al. 1995). Therefore, when antiinflammatory drugs are used around the time of mating, extra attention should be paid to uterine clearance and appropriate therapy instituted when needed.

The use of sedatives or tranquilisers may also affect uterine clearance; acepromazine decreases uterine activity (Gibbs & Troedsson 1995). Consequently, a mare being treated with acepromazine for laminitis, for example, should be monitored closely after breeding for delayed uterine clearance. Conversely, xylazine and detomidine increase uterine activity (De Lille et al. 2000, Gibbs & Troedsson

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1995); the actions of these substances may affect the response to oxytocin treatment. Some studies indicate that mares with delayed uterine clearance have different patterns of uterine activity and respond to exogenous drugs in a different manner than normal mares (vonReitzenstein et al. 2002, De Lille et al. 2000). When sedation is required in a mare in which uterine clearance is a concern, xylazine or detomidine are preferred over acepromazine because of their enhancement rather than inhibition of uterine motility and clearance.

The successful management of susceptible mares may also include uterine lavage and/or intrauterine antibiotic infusion. Regardless, the emphasis should be on treatment in relation to breeding and not ovulation. Too often in the past, veterinarians have waited until ovulation before treating these mares. By then, there has usually been a large accumulation of fluid, and the bacteria are in a logarithmic phase of growth.

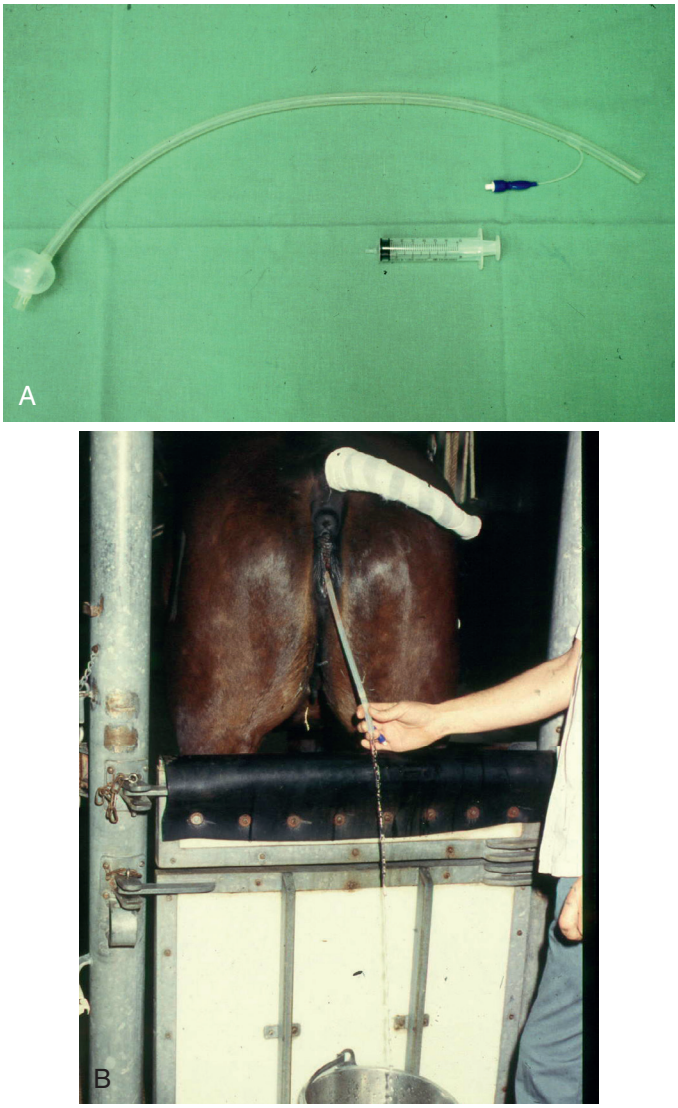
Recognition of the importance of the mechanical evacuation of uterine contents accounted for the introduction of large volume uterine lavage. The technique involves the infusion and retrieval of multiples of 1 L of warm, sterile, physiological (buffered) saline or lactated Ringer's solution into the uterus via a catheter that has been retained within the cervix via an inflatable cuff. The most convenient is a large bore (30 French) (80 cm) autoclavable equine embryo flushing catheter (Fig. 31.20A and B). The cuff is useful as it effectively seals the internal cervical os. The catheter should only be inserted after thorough cleansing of the perineum. The rationale for such an approach is:

- The removal of accumulated uterine fluid and inflammatory debris that may interfere with neutrophil function and the efficacy of antibiotics;
- Stimulation of uterine contractility; and
- Recruitment of fresh neutrophils through mechanical irritation of the endometrium.

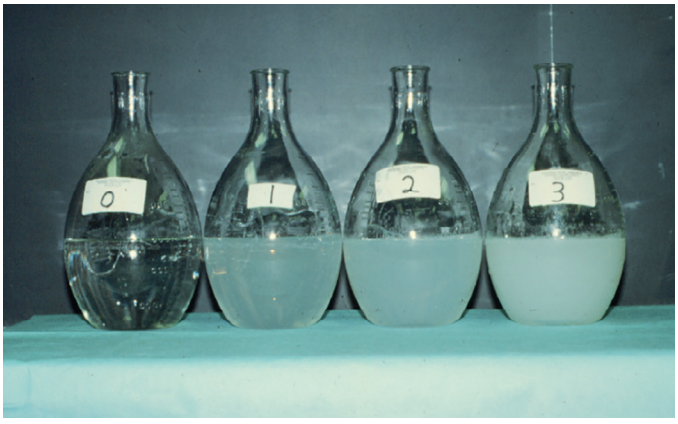
The saline is infused by gravity flow 1 litre at a time, and the washings are inspected to provide immediate information concerning the nature of the uterine contents (Fig. 31.21). The lavage should be repeated until the fluid that is recovered is clear. In most cases, the fluid is evenly distributed in both horns, making transrectal massage of the uterus unnecessary. If a rectal examination is performed while the catheter is in the uterus, care must be taken to avoid contaminating the catheter. The fluid can be recovered in the same container from which it was infused, thereby preventing air being aspirated into the uterus via the catheter. Measurement of the recovered fluid and ultrasonographic examination of the uterus should be performed after flushing to ensure that all the fluid has been recovered. This is necessary because the mare under treatment has an impaired ability to drain its uterus spontaneously. For this reason, the process is usually combined with oxytocin treatment. Ideally, these mares will be bred only once, but if repeated matings are necessary, uterine lavage should be performed after each mating. Although in many cases uterine lavage is performed at 24 hours postmating, there is some evidence that early lavage at 6 to 8 hours postmating is beneficial in problem mares (Knutti et al. 2000).

Large volume lavage is beneficial in many cases, particularly in a mare with a relatively large (more than 2 cm depth) accumulation of fluid after breeding. The process is time consuming, and there is the possibility of further contamination of the uterus by passage of a drainage tube. Nonetheless, when there is more than 2 cm of uterine fluid or a mare is known to be highly susceptible, the risks are outweighed by the benefit of treatment (Knutti et al. 1997).

It has been shown that saline lavage and uterotonic drugs are as effective as antibiotics in eliminating bacteria from the uterus



• **Fig. 31.20** (A) A large bore catheter with an inflatable cuff, used for uterine lavage. (B) Fluid drainage from the mare.



• **Fig. 31.21** Increasing grades of opacity of fluid recovered after uterine lavage.

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(Troedsson et al. 1995). However, this was an experimental study in which a single bacterial species was infused into the uterus and lavaged within 12 hours of mating. Clinically, there is often a mixed bacterial flora, and lavage cannot always be performed within 12, or even 24, hours. This is why many practitioners continue to use intrauterine antibiotics as part of the treatment protocol.

It is particularly important to recognise and manage appropriately the older maiden mare, as in many cases these mares are susceptible to postbreeding endometritis even though they have never been bred before. Often sport or Warmblood mares are not considered for breeding until they are in their teens, and these older maiden mares can be very difficult to get in foal. Many of these mares have some common characteristics. Endometrial biopsy samples reveal glandular degenerative changes and stromal fibrosis (endometriosis) as an inevitable consequence of ageing, despite the fact that they have not been bred (Ricketts & Alonso 1991a). In fact, some feel that endometriosis is worse in a mare that has never been pregnant than in a mare of similar age that has been pregnant nearly every season. Another common characteristic of these mares is uterine fluid accumulation. Often, an older maiden mare has an abnormally tight cervix that fails to relax properly during oestrus so that fluid is unable to drain and accumulates in the uterine lumen (Pycock 1993). In many cases, this fluid is negative for bacterial growth and presence of neutrophils. Once the mare is bred, the fluid accumulation will be aggravated due to poor lymphatic drainage and impaired myometrial contraction compounded by the tight cervix. The amount of intrauterine fluid will vary in individual mares, ranging from a few millilitres to over a litre in extreme cases. All too often, owners assume that the fertility of these mares is comparable to that of young maiden mares. One of the most important aspects of breeding the old maiden mare is to make the owner aware that there is a high possibility that she will be a problem. These mares must be considered highly susceptible and managed accordingly.

From 4 hours postbreeding, uterine lavage can be performed in mares with a significant fluid load. If a tight cervix is encountered, then manual cervical dilation is performed. Bader (1982) demonstrated that sperm arrived at the oviduct by 2 hours postinsemination, significantly more than after 4 hours. Brinsko and co-workers (1991) demonstrated that uterine lavage performed 4 hours after insemination did not adversely affect pregnancy rates, whereas the pregnancy rate was reduced when the lavage was performed 0.5 or 2 hours after insemination (Brinsko et al. 1990). The authors have lavaged mares within 2 hours postinsemination and achieved pregnancies, as did Brinsko and co-workers (1990); however, in view of Bader's work (1982), lower sperm numbers are likely to be present in the oviduct. It is also important to note that this work was not performed in a group of susceptible mares, and it is possible that these mares also have delayed sperm migration.

Given that breeding is not necessarily a sterile process and the ejaculate itself will likely to have a bacterial load, we need to ask the question: are antibiotics indicated in the management of the susceptible mare? The evidence in support of routine prophylactic antibiotic use is weak (Cooke 2014), despite Pycock and Newcombe (1996c) demonstrating a beneficial effect of intrauterine antibiotics and oxytocin on pregnancy rates. The authors acknowledge that a single dose of antibiotics given in this manner needs further consideration given the current state of bacterial antibiotic resistance.

The use of corticosteroids has been advocated in mares with excessive inflammation. Buccal and co-workers (2008) studied the use of 50 mg dexamethasone at the time of breeding and demonstrated an improved postbreeding uterine environment with decreased endometrial oedema and reduced fluid accumulation. Another study using a lower dose, 10 or 20 mg dexamethasone, did not show a beneficial effect on pregnancy rates (Vandaele et al. 2010). The authors have had success using 40 mg dexamethasone administered at the postbreeding examination, especially in cases in which excess uterine oedema has been identified; however, the authors' preferred choice of steroids for preemptive treatment is 0.5 mg/kg prednisolone administered twice daily, starting 24 to 36 hours before breeding, and finishing postovulation (adapted from Dell'Aqua et al. 2006).

Management Protocol Useful in the Highly Susceptible Mare. A mare that from past experience/history is known to produce a large amount (several centimetres depth) of luminal fluid after mating should be managed using the following protocol. Overall management of such mares must be of the highest quality before breeding. Good hygiene at foaling is essential, and all mares should be thoroughly examined postpartum for the presence of trauma that might compromise the physical barriers that prevent uterine contamination. Gynaecological examinations, particularly of the vagina, should be performed as aseptically as possible. Thorough digital examination of the cervix can identify fibrosis, lacerations, or adhesions that may need treatment before breeding. Because air in the vagina can cause irritation of the mucosa, it should be expelled by applying downward pressure with the hand through the rectal wall. Attention should be paid to hygiene at mating by using a tail bandage and washing the mare's vulva and perineal area with clean water (ideally from a spray nozzle which avoids the need for buckets). Breeding should occur at the optimal time, and the number of breedings should be minimised. This means that these mares need very close monitoring of the oestrous period by rectal palpation and ultrasonography. The use of hCG or other ovulation inducing agents is strongly recommended in an attempt to ensure they are bred only once. Prediction of ovulation can also be made easier by not breeding these mares too early in the year, i.e., before they have begun to cycle regularly. If feasible, the use of artificial insemination can be helpful to reduce (but not eliminate) the inevitable postbreeding endometritis.

The management protocol is as follows:

- A single breeding must be arranged 1 to 2 (or even 3) days before the anticipated time of ovulation.
- Ultrasound examination of the uterus 4 to 12 hours after mating is performed to assess the amount and echogenicity of any intrauterine fluid.
- Uterine lavage is performed 4 to 8 hours postmating.
- Oxytocin (10 IU before ovulation, but increased to 20–25 IU postovulation) should be given after lavage and approximately every 4 hours after until evening and repeated the next morning by either the intravenous or intramuscular route. In mares with lymphatic stasis the slower release prostaglandin (cloprostenol 500 mg IM) may be useful in addition to oxytocin. Cloprostenol should be given some 2 to 4 hours after the oxytocin injection but should not be used after ovulation.
- The mare is reexamined the next day, and oxytocin treatment is repeated if fluid is still present. If fluid accumulation persists, an infusion of antibiotics or another lavage procedure followed by antibiotic infusion may be performed. Evaluation of the uterus postbreeding is a crucial time to assess all mares, and too many clinicians fail to do this.

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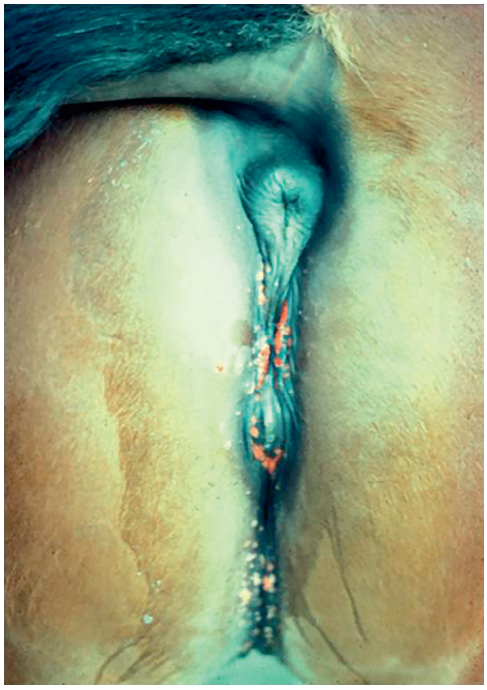

• **Fig. 31.22** Equine herpes virus 3 lesions in the perineal area of a mare.

Viral Infectious Disease

Equine coital exanthema (ECE) is a relatively benign venereal disease of both sexes caused by equine herpesvirus type 3 (Studdert 1974). It is spread by direct contact, and there have been reports of its transfer during gynaecological examinations. Once infected, horses remain carriers for life. The virus can remain dormant until conditions favour its recrudescence and proliferation with the development of the characteristic clinical signs. Normally, after coitus, signs develop after an incubation period of 4 to 7 days. Multiple vesicles appear on the vulvar mucosa and perineum, resulting in a short period of local irritation. These rupture, leaving small ulcers 3 to 10 mm in diameter that are painful to the touch (Fig. 31.22). In the absence of infection with opportunist pathogens, healing occurs in 10 to 14 days, when it ceases to be contagious; there is permanent loss of pigmentation at the site of the healed lesions. Pregnancy rates are not reduced; however, there is often reluctance to allow mating due to pain associated with the lesions. In the stallion, the vesicles develop on the shaft of the penis and the prepuce; if severe, he may be reluctant to breed.

As with other herpesvirus-induced diseases, lifelong infection is the rule. Recrudescence after stimuli such as stress, systemic disease, or trauma to the genital area can occur. No specific treatment other than disinfection of pustules and ulcers to prevent secondary bacterial infections during the acute phase is recommended. Natural service should be avoided while active lesions are present to prevent transmission of the disease. Affected mares can be artificially inseminated during the symptomatic stage of the disease or wait for 6 weeks after complete healing of ECE-associated lesions before natural service.

Protozoal Infections

Trypanosoma equiperdum causes a venereal disease called dourine, which is currently prevalent in Africa, the Middle East, and Central and South America; it has been eradicated from Europe and North America. The incubation period is 1 to 4 weeks, and the disease has an extremely protracted course that can extend over a period of weeks or months. It affects horses, mules, and donkeys of either sex. The initial sign is a nonpainful swelling of the external genitalia of both stallions and mares; mares show a vaginal discharge and stallions develop a paraphimosis. Some weeks later, depigmented areas and urticaria-like raised plaques 2 to 10 cm in diameter appear over the body surface. The disease is characterised by a low morbidity but a high mortality of 50% to 75%.

Diagnosis of dourine is based on the clinical signs, particularly the skin plaques, together with demonstration of the trypanosome in the discharges and in the skin lesions. A complement fixation test is also available. Treatment using quinapyramine sulphate has been attempted, but stallions that recover may become carriers. Therefore strict screening using a complement fixation test, with slaughter of positive and affected animals, as well as the institution of quarantine programmes, should be used to control this disease.

Infertility Classified by Anatomical Location

To maintain fertility, the uterus must be protected from the external environment. Three physical barriers exist to protect the uterine lumen. The first and most external are the vulvar labia. The second, and arguably most important, is the vaginal/vestibular sphincter; the third and last is the cervix. Poor perineal conformation is a common cause of infertility and should not be ignored in the examination of a mare with chronic endometritis, failure to conceive, or abortion due to placentitis. If a deficiency is noted, surgical correction is usually necessary to restore normal function.

Vulva and Perineum

The integrity of the vulvar labia and their anatomical relationship with the perineal area and anus are an essential component of a mare's fertility because they provide the first barrier to contamination between the external environment and the uterus. The endocrine patterns associated with each stage of the oestrous cycle and pregnancy can influence the disposition of the vulva, affecting vulvar length and tone. At least 75% of the length of the vulva should be ventral to the brim of the pelvis (the brim of the pelvis is easily palpated using the index and second fingers placed on either side of the vulva to locate the pubic bones – ¾ of the vulva should be below the fingers) (Fig. 31.23). The vulva's longitudinal axis should be perpendicular to the ground (estimate the slope of the vulva in relation to the vertical axis, it should not be greater than 10°; the greater the divergence, the poorer the conformation), and the vulvar labia should form a tight seal (perform a 'windsucker' test – gently part the labia and listen for an inrush of air) (Fig. 31.24). Absence (natural or acquired) of a normal perineal conformation can facilitate the entry of air (pneumovagina, also called 'windsucking'), faeces, and potential pathogens into the reproductive tract, which will jeopardise the mare's fertility. The initial vaginitis may lead to cervicitis and acute endometritis resulting in subfertility. In addition, pneumovagina, by creating an air filled void, disrupts the normal positioning of the tissues relative to one another with the vestibule and urethral opening, being displaced cranially. In some instances this may result in urine pooling in the vagina (urovagina). The more severe conformational abnormalities are more likely to result in failure of the vulvar seal and increased faecal contamination because the vulva forms a shelf on to which faeces may collect.

Severe loss of body condition, as experienced by some pregnant mares inadequately fed especially in winter, results in a sunken

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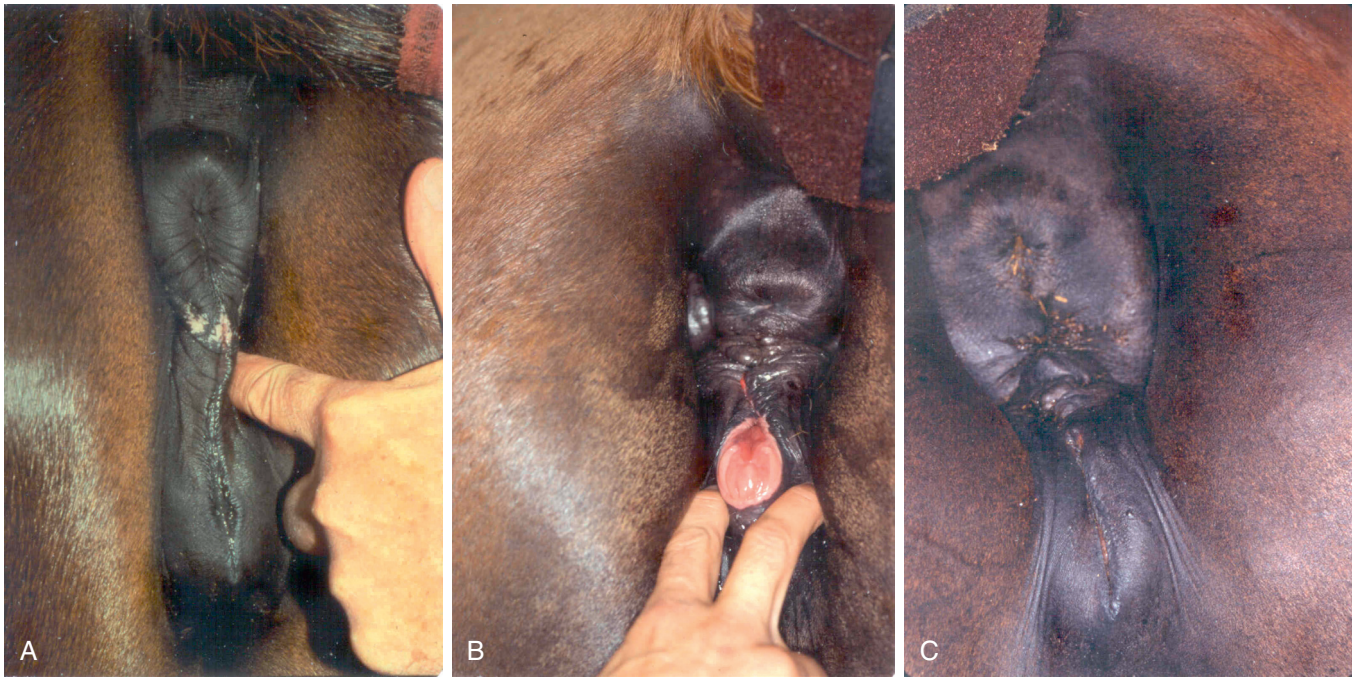

• **Fig. 31.23** (A) Location of the brim of the pelvis in a mare with good conformation; (B) location of brim of pelvis in mare with poor conformation; and (C) mare with severely sloping vulva.

• **Fig. 31.24** Parting the vulvar labia ('windsucker' test) to test the integrity of vaginal–vestibular sphincter.

anus and an increased slope of the vulva. An apparently normal perineal conformation noted in mares in good body condition may become less than adequate if body weight loss is extreme, especially in late gestation as the heavy uterus pulls the caudal reproductive tract cranially and ventrally. Contamination of the vagina with faeces during mid to late gestation may lead to an ascending bacterial placentitis, one of the leading causes of abortion and neonatal septicemia.

Ageing of the mare, associated with repeated foaling, can cause stretching and loss of tone of the perineal muscles that enable the vulva to form a barrier to external contamination and entry of air into the vagina. This condition is additionally aggravated when a mare is injured during foaling, resulting in loss of vulvar tone and apposition of the labia. Mares undergoing episiotomy may also have permanent damage to vulvar structures.

Caslick (1937) first pointed out the importance of this defect in relation to genital infection. Some breeds may be more predisposed than others. Rarely, defective vulvar conformation can be congenital or acquired due to: (1) vulvar stretching after repeated foaling; (2) injury to perineal tissue; or (3) poor body condition (old, thin mares).

Older multiparous mares are more commonly affected with pneumovagina. However, young mares that are in work and have little body fat and/or poor vulvar conformation can develop pneumovagina. In some mares, pneumovagina may only occur during oestrus when the perineal tissues are more relaxed. A 'Caslick index' has been described in an attempt to determine which mares require treatment (Pascoe 1979), but its use is not widespread. Rectal palpation of a ballooned vagina or uterus from which air can be expelled confirms the diagnosis. Cytological and histological examination of the endometrium may demonstrate significant numbers of neutrophils indicative of an endometritis. Eosinophils may also be found in association with pneumovagina.

Caslick's Vulvoplasty

This is the most common surgical procedure performed in stud farm practice. Surgical closure of the dorsal part of the vulvar labia is intended to correct poor perineal conformation. By decreasing the length of the vulvar cleft, the likelihood of the entry of air and potential pathogens into the vagina is decreased in a mare otherwise susceptible to pneumovagina, faecal contamination, and associated complications. This procedure, however, is overutilised in mares in which such intervention is not warranted. A mare with successive Caslick's surgeries can experience considerable loss of vulvar tissue, thus, paradoxically, resulting in an abnormal perineal conformation. The operation should be reserved for mares with a true vulvar defect rather than just because the mare has ovulated after mating or has failed to become pregnant.

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Another indirect complication associated with the Caslick's vulvoplasty is the increased incidence of vulvar lacerations and dystocia in which the vulva was not 'opened' by a surgical incision before foaling. Regardless of the originating cause, the Caslick's operation is used to correct first degree perineal lacerations in which only the perineal skin and vulvar mucosa are affected. For repair of second degree lacerations (laceration of deeper tissues of the perineal body) and third degree (a defect resulting in communication of the ventral rectum with the dorsal vagina) lacerations, more elaborate reconstructive surgery is required (Trotter & McKinnon 1988). This is described in the mare and cow in Chapter 20. As a rule, any postpartum corrective surgery of the perineal body and vulva should be delayed until inflammation and oedema of the involved tissues is no longer present. Mares with third degree rectovestibular lacerations invariably develop endometritis, but endometrial biopsy of mares affected with third degree lacerations have shown a rapid endometrial response after surgical repair of the laceration; mares can be then artificially inseminated as early as 2 weeks after surgery (Schumacher et al. 1992); natural breeding however needs to be avoided for several months until the tissue has healed.

Caslick's procedure is: The mare should be suitably restrained, and her vulva thoroughly cleaned and dried. The level of the floor of the pelvis is determined, which enables the surgeon to ascertain the level to which the dorsal commissure of the vulva must be sutured. Beginning at this level, the mucocutaneous junction of the vulva is infiltrated with local anaesthetic through a 21-gauge, 1-inch needle. It is important to use sufficient local anaesthetic, and in many cases at least 20 ml will be needed. The distension induced by the volume of local anaesthetic helps to evert the mucocutaneous junction. Both sides of the vulva are infiltrated in a stepwise fashion proceeding dorsally up to the dorsal commissure, being sure that local anaesthetic is placed right at the dorsal extent of the vulva. For mares operated on previously, it is important to infiltrate deeply. Using rat-toothed forceps and scissors, a *very thin* (no more than 4 mm) strip of mucosa should be removed from the anaesthetised area. For older mares that have had the operation performed several times, more radical dissection may be necessary before healthy (bleeding) tissue is reached. In some cases in which there is not much vulvar mucosa remaining, it is best just to debride the junction with a scalpel blade to induce slight haemorrhage and not actually remove any further tissue. An alternative method, that avoids removal of tissue, is to incise with a scalpel, to a depth of a few millimetres, the vulvar labia at the mucocutaneous junction in a plane parallel to the labia. The exposed submucosal tissues are sutured together using simple interrupted or continuous sutures or a locking pattern. The gauge of the suture material should not be too thick as this encourages faecal material to attach to the sutures. Skin staplers can be used, but the authors have not found it quicker compared with conventional suture techniques, and it is harder to obtain a good seal. Antibiotics are not given, but tetanus prophylaxis is needed if the mare is not adequately vaccinated.

The aim of the operation is to reduce the vulvar aperture and so prevent pneumovagina and faecal contamination of the vestibule. The time of suture removal is not crucial, and is normally done approximately 2 weeks after surgery. However, the vulva must be reopened by performing an episiotomy before the next foaling; otherwise, major injury can result. Mares that require natural mating subsequently may also need to have the Caslick's opened if the size of the vulva has been greatly reduced. If not, the vulva may tear and/or injury to the stallion's penis may occur. The episiotomy wound should be repaired soon after foaling or mating to prevent pneumovagina. If there has been severe trauma to the vulva at foaling, it may be necessary to wait for the tissue swelling to subside before attempting repair.

If repeated episiotomy followed by closure is not done very carefully, there can be considerable loss of vulvar tissue, poor healing, and major problems for the mare.

Neoplasia

Melanoma is the most common disease affecting the vulva. It occurs in 80% to 100 % of adult grey horses and, less frequently, in ageing horses of other colours. Common sites for melanoma include the anus, perineum, and vulva; there is no effective systemic treatment available for treating melanomas. For solitary benign lesions, excision may be curative. The evidence for a positive benefit for the use of cimetidine in managing melanocytic tumours is weak (Goetz et al. 1990); however, developments in the use of molecular vaccines are promising. Oncept (Merial Limited, Duluth, USA) is a canine vaccine that targets tyrosinase, a catalyst in the synthesis of melanin that is overexpressed in equine melanocytic tumours. Lembcke and co-workers (2012) demonstrated its safety and a significant positive humoural and cell-mediated immune response subsequent to vaccination with Oncept. Large scale studies to evaluate the usefulness of this approach in the clinical management of equine melanocytic tumours are ongoing.

Occurrence of squamous cell carcinoma is less common than melanomas. Squamous cell carcinoma of the vulva is observed more commonly in the lower latitudes where solar radiation is more intense. Genital tumours are at risk of metastatic spread to local lymph nodes. Lesions may be removed by standard surgical techniques, laser surgery, or cryosurgery; however, regrowth is likely to occur if surgical margins are not adequate.

Clitoris

The clitoris is enclosed in the clitoral fossa in the ventral part of the vulva but is rhythmically exposed after urination or teasing (winking) as a result of contractions of the vulvar constrictor muscle. The clitoris has several sinuses in which is found a natural smegma. Whereas the lateral sinuses are shallow, the median sinuses are deep enough to allow the growth of bacteria. Understanding the anatomy of the clitoris of the mare has become particularly important because it is an important reservoir for bacteria that may have infected the reproductive tract; of particular note is *Taylorella equigenitalis,* which causes contagious equine metritis (CEM). The disease is highly contagious, and the organism can be harboured in the clitoral fossa and sinuses (especially the median sinuses) for prolonged periods. The same applies to pathogenical strains of *Pseudomonas aeruginosa* and *Klebsiella pneumoniae.*

Even in CEM-free areas, it is important to keep in mind that the clitoral sinuses may function as a nidus for uterine infections, especially those iatrogenically induced during diagnostic procedures of the reproductive tract or artificial insemination. Careful cleaning of the perineal and vulvar areas, including the clitoral fossa, with a mild disinfectant before any invasive procedures are performed minimises the risk of introducing potential pathogens into the uterus.

Congenital anomalies of the external genitalia are seen in intersex animals, which may have underdeveloped vulvar labia associated with an abnormally enlarged clitoris. Treating prepubertal mares with anabolic steroids may lead to enlargement of the clitoris, resulting in a partially and permanently exteriorised clitoris.

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Vagina

The vestibule is the area of the genital tract that separates the vulva and clitoris from the vagina. At the cranial border of the vestibule, where it meets the vagina, lies the vaginovestibular fold or sphincter. This folded mucous membrane acts as the second and most important (Hinrichs et al. 1988) physical barrier between the uterus and the external environment. In young horses the hymen is usually a weak membranous extension of the vaginovestibular fold; occasionally, a persistent hymen may be present in a maiden mare. Usually, a manual examination of the vagina will be enough to rupture the persistent hymen; some mares with a persistent hymen may accumulate fluid in the vagina proper and uterus (mucometra). Sometimes the hymen may be so tough that it can only be ruptured using a guarded scalpel blade or scissors. The small incision can then be enlarged using the fingers and hand; once the persistent hymen is disrupted, evacuation of the fluid is uneventful. Rarely, failure of proper fusion of the Müllerian ducts may result in the presence of dorsoventral bands of fibrous tissue in the anterior vagina and fornix. They do not necessarily interfere with fertility and are often easily broken down manually.

Listening for an inrush of air into the vagina when the vulvar labia are gently parted can test the adequacy of the vaginovestibular fold as a physical barrier to external contaminants. A positive test (noticeable the sound of air rushing into the vagina) indicates that the vestibular fold is not adequately protecting the vagina proper from the outside environment.

Improper functioning of the first barrier (vulva) and second barrier (vaginovestibular fold) may lead to the constant or frequent entry of air into the vagina. The condition can be exacerbated during oestrus, when the perineal body is more relaxed than in other stages of the oestrous cycle. Accumulation of small amounts of a frothy fluid in the cranial vagina are indicative of pneumovagina, as well as the presence of air in the uterus (pneumouterus) and can be identified as hyperechoic particles present between the endometrial folds during ultrasonographic examination.

In mares with severe alterations of the perineal conformation, vulvar closure using Caslick's vulvoplasty may not correct the defect and surgical reconstruction of the perineal body (perineoplasty) may be required.

Urovagina

This is also known as vesicovaginal reflux or urine pooling and refers to the presence of urine in the cranial vagina and possibly in the uterus. As with pneumovagina, mares with marginally normal perineal conformation may be predisposed to the accumulation of urine in the vagina during oestrus when reproductive organs and the perineal body are relaxed. Transient urine pooling, which is sometimes found in postpartum mares, usually resolves after uterine involution has occurred. In older mares with splanchnoptosis (the abnormal downward displacement of one or more internal organs), the reflux of urine into the genital tract may be permanent. Clinical signs can include urine dripping from the vulva, urine scalding, and a history of failure to conceive. Diagnosis is easiest using a speculum examination during oestrus to detect urine in the cranial vagina. Urovagina can cause vaginitis, cervicitis, and endometritis, which ultimately result in infertility. The condition is surgically corrected by vaginoplasty (perhaps more correctly termed caudal relocation of the transverse fold, as surgical intervention is in the vestibule) (Monin 1972), urethral extension (Hughes & Loy 1969), or perineal resection (Pouret 1982).

Varicose Veins

During oestrus and especially during pregnancy, varicose veins may develop in older mares. Varicose veins can be present in any part of the vagina; however, they are often found in the vestibular/vaginal area. Bleeding may occur after natural service, or spontaneously during mid to late gestation. Occasionally, a persistent haemorrhage will result in considerable blood loss; however, the condition usually subsides with the end of pregnancy. Cautery or ligation of varicose veins is warranted if haemorrhage is persistent or frequent.

Neoplasia

Vaginal neoplasias are uncommon in mares. Leiomyomas and squamous cell carcinomas have been reported.

Rectovaginal Fistulas

During parturition the foal's foot may be directed towards the dorsal vagina/ventral rectum, and if unattended, a third degree perineal laceration will follow. However, if the dystocia is corrected in time, the damage may be limited to a first or second degree rectovaginal laceration or fistula. The condition is most often seen in young, primiparous mares, although the overall occurrence is less than 0.1% of all foalings. Immediately after it has occurred, it is usually difficult to estimate the amount of damaged and devitalised tissue present, even though the edges of the wound may look fresh and clean. This is why immediate repair is not normally performed unless it is possible to attempt repair within 2 hours of the injury; however, most clinicians advise delaying surgery (see Chapter 20).

First aid treatment should include:

- Debridement of nonviable tissue;
- Provision of haemostasis and general cleaning of the area;
- Parenteral broad spectrum antibiotics for 5 days;
- NSAIDs and tetanus prophylaxis;
- Daily cleaning; and
- Monitoring of uterine involution.

Originally, it was thought that these lesions should be corrected after surgical conversion to a third degree perineal laceration, In addition, it was recommended that this was delayed by a minimum of 6 to 10 weeks postfoaling; often they were left until after weaning, and the mare was left barren that season. More recently, Jalim and McKinnon (2010) described a predominantly transrectal repair technique, performed immediately postbreeding; the results compared favourably to previous techniques and allowed breeding within the same breeding season in which the injury occurred. Surgical correction of the anatomical defect (described in Chapter 20) is necessary to restore fertility.

Breeding Trauma

Vaginal lacerations may occur during natural service when the stallion is disproportionately large compared with the mare. It is important to consider the location of a vaginal laceration regarding treatment and prognosis. Based on the location of the tear in relation to the peritoneal reflection, a vaginal tear may communicate with the peritoneal cavity, likely resulting in peritonitis, or it may be retroperitoneal. Treatment includes broad spectrum antibiotics, antiinflammatories, and tetanus prophylaxis. Peritoneal lavage may be beneficial if the tear communicates with the peritoneal cavity. A breeding roll positioned under the mare's tail and dorsal to the stallion's penis will prevent the stallion from introducing its penis's full length into the vagina and, consequently, help to preventing mating-induced trauma.

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Cervix

The cervical injuries and abnormalities are often overlooked as a possible cause of infertility. The cervix is the last of the three physical barriers protecting the uterus from the external environment; cyclic hormonal changes determine the tonicity of the cervix. Anatomically, the cervix is a thick-walled sphincter, with dilation and contraction occurring as a consequence of the action of the longitudinal and circular smooth muscle, which is also rich in elastic fibres. Distinctive features of the equine cervix are its dilatability and the absence of rigid, annular constricting rings as seen in ruminants. During anoestrus, the cervix is flaccid and dry and may be partially open. Higher concentrations of progesterone during dioestrus or pregnancy cause the cervix to become tubular, firm, and tightly closed. These changes are readily palpable per rectum. Moreover, a vaginal examination during oestrus will reveal the cervix positioned low upon the ventral floor of the cranial vagina, relaxed, and easily dilated or open, thus allowing access to the uterus. The cervix must relax during oestrus to allow intrauterine ejaculation and drainage of uterine fluid. During dioestrus or pregnancy, a vaginal examination will reveal a tightly closed cervical os, which is pale in colour and positioned dorsal to the floor of the vagina. In older mares or those with fibrotic changes due to cervical trauma, failure of cervical relaxation can result in delayed uterine clearance or persistent mating-induced endometritis. Artificial insemination has been used successfully in mares with an abnormally narrow cervix. Failure to relax can be treated with local application of PGE and/or manual dilation. Interestingly, mares with a fibrous cervix that become pregnant do not normally have any difficulties at foaling. Failure of the cervix to close during dioestrus can lead to persistent endometritis and failure to conceive or early embryonic death; failure to maintain closure during pregnancy can lead to gestational failure. Both surgery and exogenous progesterone have been used in an attempt to encourage closure of the cervix.

Assessment of the cervix must form a part of the routine prebreeding examination of a mare, either directly using a speculum per vagina and/or by digital exploration, preferably during dioestrus when it is more tightly closed under the influence of progesterone.

Cervicitis

Inflammation of the cervix often accompanies vaginitis or endometritis. Cervicitis is most commonly seen in the postpartum period, especially when there has been dystocia. Severe cervicitis associated with metritis may be also seen in mares infected with organisms such as *Taylorella equigenitalis* that cause a copious purulent discharge (Katz et al. 2000). Infusion of certain chemicals, such as chlorhexidine or strong iodine solutions, into the uterus to treat endometritis may irritate not only the endometrium but also the cervix and vaginal mucosa. If potentially irritant solutions are used, vaginal speculum examinations between treatments to assess the condition of the cervix are warranted.

Trauma

Although cervical lacerations may occur during natural service, they are usually small and resolve without major consequences. Occasionally, maiden mares in oestrus have a tightly closed cervix, which may suffer laceration during natural service, especially if the stallion is disproportionately larger than the mare or the mare was not in full physiological and behavioural oestrus. However, these tears are usually small and heal without further treatment. Most serious lacerations occur during parturition and may occur during normal parturition or can be iatrogenic during intervention to correct a dystocia by mutation or fetotomy (see Chapter 20). Although the cervix should be examined digitally after dystocia, especially if a fetotomy is involved, the extent and severity of a cervical laceration is best evaluated once the cervical lesion is healed. The competency of the cervix should be evaluated during dioestrus or when the mare is under the influence of exogenous progestogens, thus verifying its ability to close tightly. The cervix can also be permanently damaged by irritant chemicals such as povidone iodine. Adhesions of the cervix can be broken down manually, but this must be done daily to prevent recurrence. If severe, adhesions may contribute to the development of pyometra; older, multiparous mares may be predisposed to cervical lacerations (Miller et al. 1996).

Transluminal adhesions and anatomical defects are often more easily diagnosed by digital examination of the cervix rather than by vaginoscopy. There are differences of opinion on which cervical lacerations require repair. If severe, cervical lacerations may need surgical repair to restore normal cervical shape and function, but because surgical correction is difficult and not always rewarding, a biopsy of the uterus should be taken to assess the mare's potential to maintain a pregnancy before surgical correction is attempted. Repairs can be performed in the standing patient or in the Trendelenburg position for ventral tears (O'Leary et al. 2013).

Postbreeding repair of cervical lacerations has been described by Foss and others, (1994). Because many cases of cervical lacerations are only detected at the prebreeding examination or at the time of AI, postbreeding repair is preferable because it avoids a 4- to 6-week healing period, resulting in a reasonable chance of pregnancy occurring. Developmental abnormalities of the cervix have been described; these include aplasia and a double cervix.

Cervical Polyps or Cysts

Occasionally, pedunculated, cyst-like structures are found on visual or manual examination of the cervix. They are attached to either the cervical os or emanate from the cervical lumen and protrude into the vagina or uterine body. Although the cause is unknown, they do appear to be associated with infertility, and removal either by laser or ligation is recommended.

Uterus

Ventral Sacculations

Aside from changes associated with pregnancy, other pathological conditions may cause focal enlargements in the ventral portion of the uterus. These uterine changes are usually associated with increased age and parity and are invariably found at the base of either or both uterine horns where pregnancies are usually established. These enlargements may be mistaken for a pregnancy, especially if inexperienced palpators do not use ultrasonography to confirm their palpation findings. Mechanisms contributing to formation of ventral uterine enlargements have been identified, including but not limited to: endometrial atrophy, focal myometrial atonia, and lymphatic lacunae (Kenney & Ganjam 1975). Furthermore, in older and multiparous mares, the uterus may tilt ventrally in relation to the pelvic brim (uterine splanchnoptosis). Mares with ventral sacculations and uterine splanchnoptosis have a higher incidence of delayed uterine clearance than normal mares (LeBlanc et al. 1998).

Endometrial Cysts

Endometrial cysts (Fig. 31.25) are often cited as a cause of infertility; however, a cause and effect relationship has not been clearly

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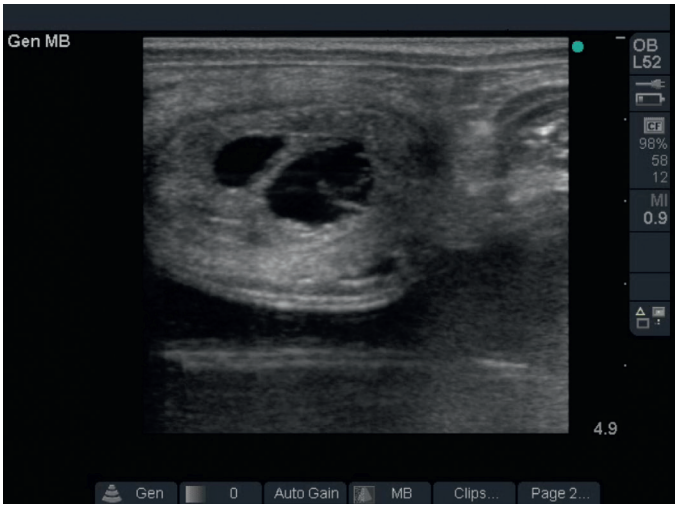
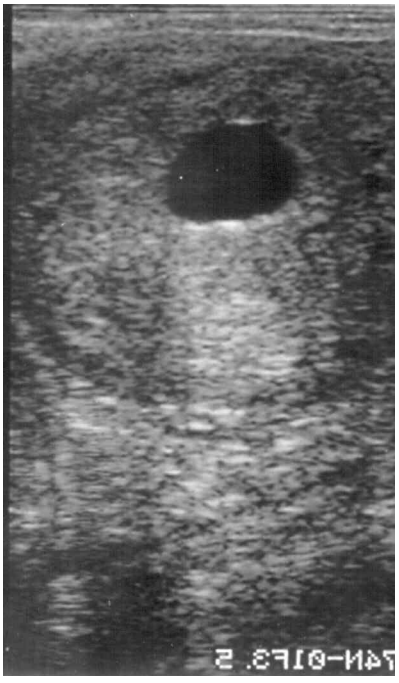
• **Fig. 31.25** Endometrial cyst, removed by stalk transection.

established. Rather than being viewed as a cause of infertility, endometrial cysts should be considered as an indication of underlying pathological changes in the uterus. Endometrial cysts are of lymphatic origin, and their occurrence may be associated with disruption of normal lymphatic function. The proportion of mares with endometrial cysts increases with age. Mares over 11 years of age are more than four times as likely to have endometrial cysts as younger mares, and a majority of mares over 17 years of age will have endometrial cysts (Eilts et al. 1995). Reports that associate endometrial cysts with a lower pregnancy rate or increased embryonic loss fail to account for the effect of advancing age and degenerative changes in the endometrium. When the effects of, for example, such factors as parity and age are considered, the assumption that cysts cause infertility is not supported (Eilts et al. 1995). Another report by a different group of researchers, which did control the time of pregnancy diagnosis, similarly found no difference in pregnancy loss between mares with cysts compared with those without, although mares with endometrial cysts had a lower day 14 and day 40 pregnancy rate (Tannus & Thun 1995). There appeared to be a quantitative effect of cysts on fertility because an effect was not evident until a mare had numerous cysts or the cysts were very large. However, even then, the effect of endometrial cysts on fertility was much less than that seen with delayed uterine clearance or intrauterine fluid accumulation. A quantitative effect of endometrial cysts could be due to interference with embryonic mobility. It is well known that the equine embryo undergoes a period of mobility after entering the uterus, finally becoming fixed at approximately 16 or 17 days gestation. If mobility is restricted during this period and the embryo is not permitted to come in contact with a sufficient portion of the endometrium, maternal recognition of pregnancy may not occur, resulting in luteolysis and embryonic loss. Additionally, if cysts are numerous and/or large at the site of embryonic fixation (at the base of the uterine horns), the presence of the cyst(s) may interfere with nutrient exchange between the endometrium and the developing conceptus, much in the same way as two opposing twins (Ginther 1989c).

Endometrial cysts are best diagnosed with ultrasonography. Cysts can be identified as hypoechoic, immovable structures with a clear border in the lumen of the uterus (Fig. 31.26), as opposed to intraluminal fluid, which is movable and has a less distinct shape or border. Endometrial cysts are usually multiple and are most commonly found located at the base of the uterine horns. Cysts may change in size and number during the estrous cycle and pregnancy.



• **Fig. 31.26** Endometrial cysts, observed on ultrasonographic examination.



• **Fig. 31.27** Endometrial cysts can appear much like a conceptus during ultrasonographic examination.

Endometrial cysts can complicate early pregnancy diagnosis because, often, an endometrial cyst can be similar in size and appearance to an early conceptus. Cysts that appear spherical can often be shown to have a more irregular shape if the ultrasound probe can be reoriented in relation to the cyst. To make early pregnancy diagnosis easier and more reliable, the size and location of endometrial cysts should be recorded either using a diagram or by storing ultrasonographic images during a prebreeding examination. Differentiation is based not only on previous cyst mapping but also the early mobility of the conceptus, the presence of specular reflections, the conceptus's spherical appearance, and growth rate. Some cysts can be very difficult to distinguish from pregnancies (Fig. 31.27), and pregnancy examination may need to be repeated or confirmation delayed in some mares with endometrial cysts.

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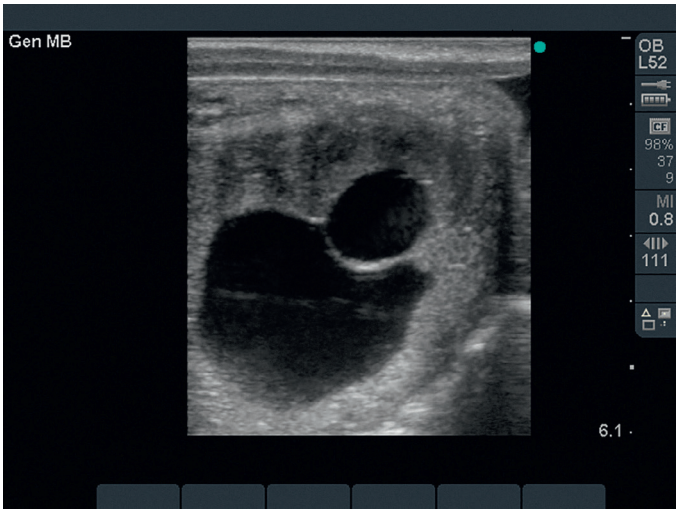
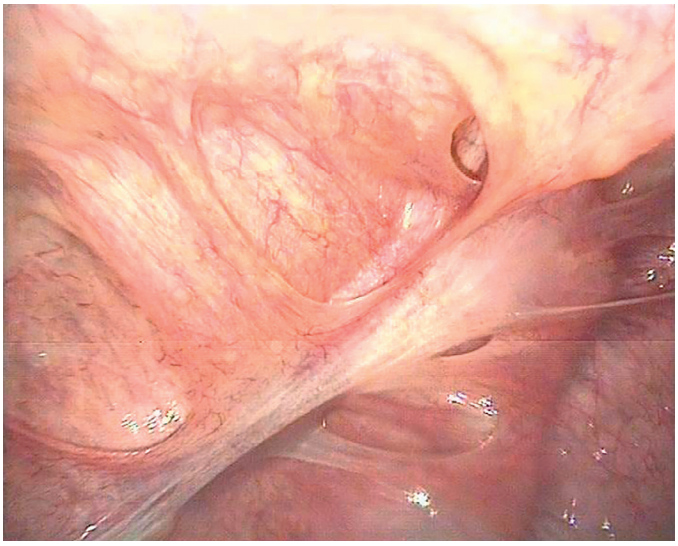


• **Fig. 31.28** Showing an endometrial cyst (small and round) and a larger 18-day conceptus.



• **Fig. 31.29** Uterine adhesions that are formed as a result of intrauterine iodine infusion.

Often, there is an opportunity to scan the mare before or immediately after breeding, but this is not always possible. In that case, it may be impossible to be certain whether there are: twin unilateral vesicles of approximately 20 and 16 days; a single pregnancy and a 19 mm cyst; a cyst with adjacent free fluid; or a 19 mm conceptus with adjacent free fluid. In these cases, it is vital to study the ultrasonographic image carefully. When one looks closely at the wall between the two structures in Fig. 31.28, it is relatively thick and hyperechoic compared with the twin vesicle shown in Fig. 31.7. This would confirm that at least one structure is a cyst. Reassessment of the irregular structure will confirm that the fluid is contained and does not extend up or down the horn as would be found with free fluid. The appearance of an embryo in the conceptus around 20 to 22 days of gestation or of a heartbeat at approximately 24 days provides a definitive diagnosis. Thorough identification of cysts at the beginning of the breeding season minimises the chance of false positive pregnancy diagnosis.

In most cases of endometrial cysts, no treatment is necessary other than recording their size and location for future reference during pregnancy examination. However, if the cysts are sufficient in size or number that they pose a potential threat to embryonic migration, treatment can be aimed at facilitating establishment of pregnancy by providing exogenous progestogen. If a mare is diagnosed at the beginning of the breeding season with a large number of cysts, it is generally best to continue to attempt to get the mare in foal that season. Progestogen, usually in the form of altrenogest (0.044 mg/kg, PO, daily), can maintain pregnancy even when the signal for maternal recognition of pregnancy is lacking and can be used in an attempt to circumvent the interference with embryonic mobility caused by the cysts. If she fails to become pregnant, some form of treatment should be attempted, and an endometrial biopsy should be taken to help determine the likelihood of her carrying a foal to term. The cysts may be removed surgically, usually transendoscopically, utilising a laser or electrocautery loop to ablate it, or in the case of the latter, snare and subsequently remove cyst tissue. Wounds after cautery appear to heal very quickly, usually within 4 to 6 weeks. Because endoscopy should be done while the mare is in dioestrus when the cervix is relatively closed, PGF should be given after cauterization, and the uterus should be lavaged with saline. Ligation and transection of the stalk of pedunculated cysts is an alternative. Merely puncturing and draining the cyst or incising its wall will not provide long-term remission.

Most uterine cysts involve the endometrium, but occasionally, an extraluminal uterine cyst lying external to the endometrium can be identified on ultrasound examination. Its location should be verified by identification of the uterine lumen; extraluminal cysts usually have no adverse effects on fertility.

Uterine Adhesions

Severe infectious or chemically-induced (after intrauterine infusion of irritating chemicals) endometritis may cause the formation of transluminal adhesions in the uterus. Uterine adhesions are most frequently diagnosed on endoscopic examination. It has been suggested that the incidence may be greater than was previously thought (Stone et al. 1991). Multiple adhesions adversely affect fertility by causing fluid accumulation resulting in mucometra or pyometra or by affecting the mobility of the conceptus. Severe adhesions can completely obstruct one or both of the uterine horns. It is possible to remove the obstruction endoscopically by either cautery or laser techniques, starting at the thin membranous parts of the obstruction (Fig. 31.29). It is important not to 'burn' too deeply the uterine wall as in these cases more severe damage can occur. After removing the obstruction the uterus should be flushed to remove any debris, and the mare treated with PGF. In addition to an assessment of an endometrial biopsy, the prognosis for future breeding also depends on the severity of the obstruction and to what extent the obstruction could be removed.

Adhesions involving the serosal surface of the uterus may occur after Caesarean section or uterine tears. No effective treatment other than attempted surgical excision exists once they are formed. Therefore steps should be taken to minimise the likelihood of their formation, including daily palpation of the uterus after Caesarean section.

Uterine Foreign Bodies

Uterine foreign bodies (e.g., fetal remnants), which may act as a nidus for the establishment of chronic endometritis, have been documented (Ginther & Pierson 1984) but are uncommon. In one case the mare had a history of failure to conceive, with short interoestrous intervals after a dystocia. Ultrasound examination

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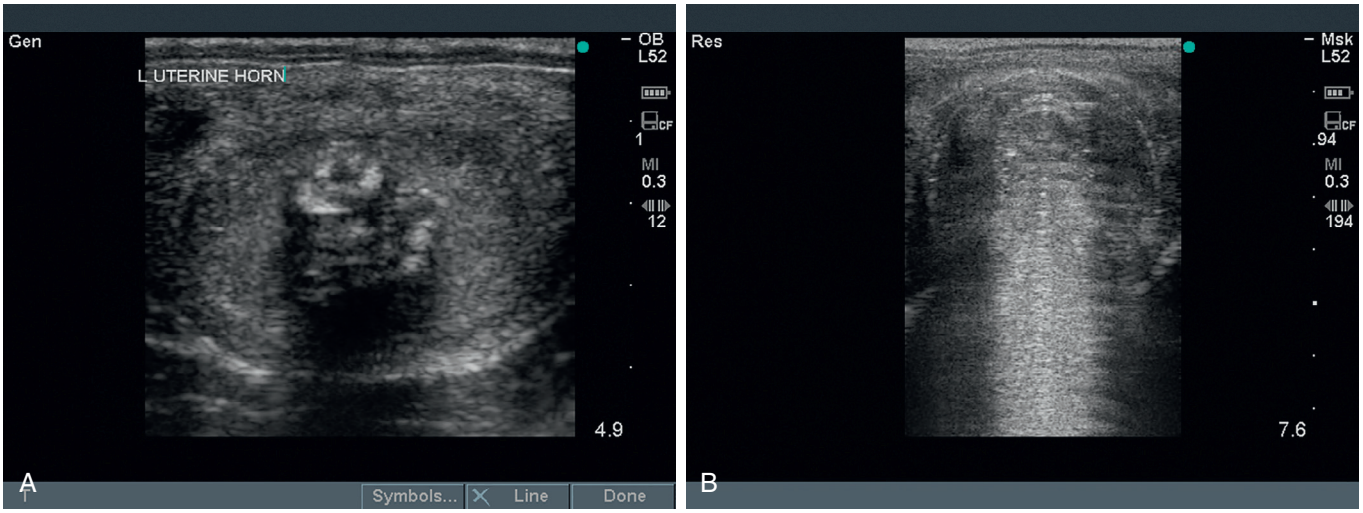
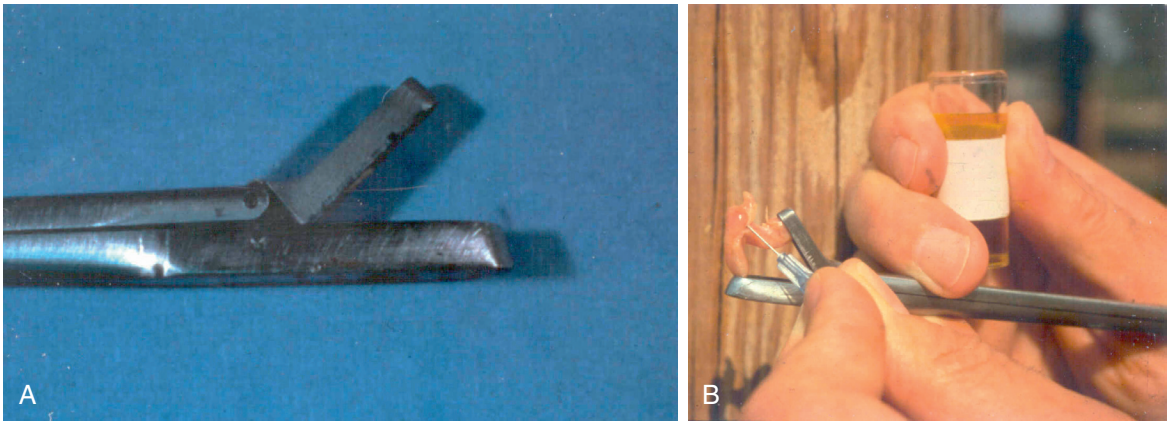


• **Fig. 31.30** (A) Hyperechoic fetal bone visible on ultrasonographic examination of the uterine body. (B) Ultrasound appearance of an intrauterine glass marble.



• **Fig. 31.31** (A) Uterine biopsy instrument. (B) Biopsy tissue being removed after procurement and then placed in Bouin's solution for fixation.

revealed hyperechoic fetal bone, part of the scapula, (Fig. 31.30A) which was removed manually via the cervix. The mare conceived after appropriate intrauterine lavage and antibiotic treatment for the endometritis. Other foreign bodies that have been reported include straws after AI and the tips of uterine swabs. More recently, the use of intrauterine glass balls (see Fig. 31.30B) for the management of oestrous behaviour in fillies and mares has led to cases of whole or fractured glass balls acting as foreign bodies with or without associated uterine infection (Turner et al. 2015, Morris et al. 2017). As a result, glass balls (marbles) are not recommended for this purpose, but rather sterile water filled or solid plastic spheres are recommended. However, it should be noted that there are no studies into the long-term presence of these devices in the uterus of mares, and therefore, if they are to be used, they should be removed at the end of the physiological breeding season.

Biopsy

Before committing to the expense of laser surgery for removal of cysts or other reconstructive urogenital surgery, an endometrial biopsy is usually recommended. An endometrial biopsy is also usually considered to be a routine part of a complete breeding soundness or end of season barren mare examination. Because an endometrial biopsy can aid in predicting a mare's chances of carrying a foal to term, the information provided by a biopsy should be considered before purchase or undertaking reproductive surgery such as repair of a cervical tear. Biopsies in some cases provide information that is useful in the diagnosis of infertility and may provide a basis for treatment. It must be realised, however, that an endometrial biopsy alone is not the only, nor usually the most important, piece of information and must be taken in context with other information obtained from the history and reproductive examination.

The procedure is as follows: The perineal area should be thoroughly cleaned for an aseptic procedure. The biopsy instrument is held in a closed position and passed manually through the cervix. Once the instrument is placed into the uterus, the hand is withdrawn from the vagina and placed in the rectum and the tip of the instrument identified. The instrument is then opened and the uterus is pressed through the jaws of the biopsy instrument and a small portion of an endometrial fold is obtained by closing the biopsy forceps (Fig. 31.31A and B).

Generally, a biopsy specimen is taken from a site at the base of one of the uterine horns. When procuring a biopsy, care should be taken that the tissue is not obtained from a site near the internal cervical os. Glands are less dense near the cervix, making a biopsy obtained from that area less representative of the uterus as

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a whole and more difficult to interpret. Moreover, if the cervix is accidentally biopsied, adhesions can result.

It has long been considered that a single biopsy is representative of the entire uterus; however, studies have shown that variation by as much as an entire category may exist between sites (Dybdal et al. 1991). Therefore a thorough examination by palpation and ultrasonography should be performed first to determine whether any areas of the uterus appear to be 'abnormal'. If an abnormal area is detected, biopsies should be obtained from both the 'abnormal' and the 'normal' areas. Repeated or multiple biopsies do not significantly affect subsequent fertility because a mare may become pregnant when bred just a few days after a biopsy specimen is taken (Watson & Sertich 1992).

Biopsies may be taken at any time during the year or during any stage of the oestrous cycle because the mare's cervix is easily dilated. Some recommend taking a biopsy during dioestrus for diagnostic purposes because the endometrium is under the influence of progesterone and the glands have achieved maximum tortuosity. Others recommend taking a biopsy during oestrus when the cervix is relaxed and the biopsy instrument is most easily passed into the uterus. Either way, it is important to relay all pertinent history, including the stage during which the biopsy was taken, to the pathologist reading the biopsy. Periglandular fibrosis may appear worse in biopsies taken during anoestrus because of the sparseness of glands. In addition, biopsies taken during anoestrus or the transition phase may have evidence of increased inflammation because the cervix has been in a relaxed state for a prolonged period due to the absence of progesterone. A recent study by Killisch and co-workers (2017) demonstrated that 22% to 30% of biopsies taken during the spring or autumn transition demonstrated 'irregular' endometrial differentiation, and they concluded that diagnostic biopsy sampling should therefore be performed between late April and before September. Thorough descriptions of the pathological changes have been published and can be reviewed, but in most cases, a pathologist associated with the service that processes the sample will read the biopsy. Even so, if the practitioner can review the biopsy slide, it can be helpful in developing recommendations for the future management of the mare.

Endometrial biopsies are classified into four categories (I, IIA, IIB, III) based on scheme published by Kenney and Doig (1986). A mare with a Category I biopsy has an essentially normal endometrium. The likelihood of her becoming pregnant and carrying a foal to term, estimated at 80% to 90%, depends more on broodmare management than on the mare's inherent fertility. Mares with a Category III biopsy have severe pathological changes in the endometrium and an estimated 10% chance of carrying a foal to term, even with good breeding management. Most mares will be classified as a Category IIA or IIB, with an estimated 50% to 80% and 10% to 50% chance, respectively, of carrying a foal to term; it is reflective of a combination of management practices and the mare's inherent fertility.

A complete histopathological description is usually provided by the pathologist. However, of primary concern to the practitioner is the severity and distribution of inflammation and degenerative changes, including periglandular fibrosis, angiosis, and lymphatic lacunae. Degenerative changes carry a worse prognosis than inflammatory changes because they are considered to be permanent and progressive. Attempts have been made to improve the 'quality' of the endometrium and improve breeding prognosis by physical (Ricketts 1985) or chemical curettage (Bracher et al. 1991). Ricketts (1985) demonstrated that mechanical endometrial curettage resulted in an overall improvement in the extent and degree of chronic degenerative endometritis in 82% of cases; 58% of the mares produced live foals after curettage. Bracher and co-workers (1991) reported on the use of kerosene as a chemical agent for endometrial curettage. An infusion of 50 ml of commercial kerosene during dioestrus induced a severe acute inflammation of the endometrium of short duration with mild to severe necrosis of the luminal epithelium. Pregnancy was established in 65% of treated mares, half of which had grade 3 histological changes; no adhesions developed in any mares treated. Although these studies suggest an improvement in biopsy grade and pregnancy outcome, the techniques have not been widely adopted. The use of kerosene remains particularly controversial, and there is a lack of information from controlled, randomised clinical trials. The cause of such degenerative conditions is not known, although it is presumed by many to be due to repeated insults to the uterus. They are more commonly seen in older mares (Ricketts & Alonso 1991a). Dilated lymphatics are often an indication of a uterine clearance problem; however, delayed clearance is more reliably diagnosed by ultrasonographic examination in the postmating period.

Although biopsy can reveal the presence of an inflammatory condition, other methods (such as examination of perineal conformation) must be employed to reveal why it is present, and a culture is needed to identify the particular pathogen. A repeat biopsy after appropriate therapy may reveal the degree of success of treatment and allow an assessment of breeding prognosis (Ricketts & Alonso 1991b).

Endometriosis

Endometriosis has been referred to in the past as chronic infiltrative endometritis, and currently refers to the presence of fibrosis in the stromal and periglandular compartments. The degree of endometriosis is closely associated with the ability of a mare to establish and maintain a healthy pregnancy until term. Parity and age contribute to degenerative changes occurring in the endometria of mares (Ricketts & Alonso 1991a).

Fibrotic changes may be seen around the endometrial glands and in association with the basement membrane in the stratum compactum. The amount and pattern of the distribution of the fibrotic tissue has been descriptively classified as: slight, 1 to 3 layers of periglandular fibrosis; moderate, 4 to 10 layers; severe, greater than 10 layers of periglandular fibrosis (Kenney 1978). Cystic glandular dilatation is another manifestation of endometriosis. Periglandular fibrosis, glandular epithelial hypertrophy, or inadequate lymphatic drainage may lead to dilatation of the endometrial glands.

Other degenerative alterations in the endometrium that lead to endometriosis include lymphatic lacunae and angiosis (Gruninger et al. 1998, Ricketts & Alonso 1991a). Lymphatic lacunae are histopathological indications of lymphangiectasia. Angiosis (vascular pathologies) is associated with ageing and parity, especially in uteri with ventral sacculations and associated venous congestion, which are pathogenical factors for angiosis (Gruninger et al. 1998). There are no treatments for these anatomical and vascular degenerative changes in the uterus. Mares with lymphatic lacunae and disseminated uterine angiosis are at risk of infertility caused by delayed uterine clearance and persistent mating-induced endometritis (Gruninger et al. 1998, Ricketts & Alonso 1991a).

Pregnancy loss attributed to endometrial fibrosis is more commonly manifested during the embryonic period, postulated as due to a decreased ability to produce histotrophe, required by the embryo for nutritional sustenance. If uterine fibrosis interferes with implantation of the placenta, then pregnancy failure will occur later in gestation. Microcotyledonary attachments begin to

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develop between 80 and 120 days of gestation (Bracher et al. 1990). Ultrastructural evaluation of the placenta in mares with chronic degenerative endometritis (endometriosis) has shown a delay in microcotyledon development and a reduction in the number of microcotyledons and villi per surface area (Bracher et al. 1991). Endometrial atrophy may not result in abortion but can influence fetal growth. Fetal weights in mares with degenerative endometritis were lower than in normal mares (Bracher et al. 1991). Evidence of endometrial atrophy may be evident upon inspection of the placenta after the birth of the foal (Asbury 1988).

Pyometra

Pyometra is an accumulation of large quantities of purulent exudate in the uterus causing its distention (Hughes et al. 1979). It must be distinguished from the smaller, intermittent accumulations of fluid that can be detected by ultrasonography in acute endometritis. Pyometra occurs because of interference with natural drainage of fluid from the uterus, which may be due to cervical adhesions or an abnormally constricted, tortuous, or irregular cervix. In some cases, the fluid accumulates in the absence of cervical lesions, presumably due to an impaired ability to eliminate the exudate.

Unlike cows, mares with pyometra do not necessarily have a persistent CL, and many may cycle normally. When the endometrium is severely damaged, resulting in extensive loss of surface epithelium, severe endometrial fibrosis, and glandular atrophy, a prolonged luteal phase, presumably due to interference with the synthesis or release of PGF, may be observed.

A mare with pyometra seldom shows overt signs of systemic disease even when there is up to 60 litres of exudate in the uterine lumen; very occasionally, there is weight loss, depression, anorexia, or recurrent colic. Pyometra has been classified into two categories in mares: open and closed (Hughes et al. 1979). In a case of closed pyometra, the fluid accumulates due to a closed cervix, whereas in open pyometra, the cervix remains partially dilated but purulent material accumulates because of impaired uterine clearance. A vulvar discharge is often observed in open pyometra, especially at oestrus, which may vary in consistency from watery to cream-like.

In a mare with an enlarged uterus, pyometra is readily diagnosed by transrectal ultrasonography when a large volume of intraluminal fluid with moderate echogenicity is seen in the uterus. Due to the lack of systemic illness, most mares with pyometra are brought to the veterinarian's attention at an advanced stage. Degenerative changes such as endometrial atrophy may preclude mares from returning to normal fertility after treatment. A biopsy of the uterus should be examined to determine the prognosis for potential fertility before treatment of pyometra.

The aim of treating pyometra is to expel the purulent material from the uterus. In the absence of systemic illness or an unsightly vulvar discharge, treatment of chronic pyometra may not be indicated, although some mares can show signs of discomfort during exercise. Many cases can be significantly improved by repeated large volume lavage with several litres of warm saline via a wide-bore tube such as a nasogastric tube. Initially, PGF can be used to induce luteolysis of the CL if present, which should allow the cervix to relax sufficiently for digital exploration for the presence of any adhesions. PGE may also help relax the cervix. After repeated large volume lavage and oxytocin to achieve drainage of exudate, appropriate antibiotics, based on culture and sensitivity, should be infused. If a cervical lesion is identified, it must be corrected to reduce the chance of recurrence of the pyometra. Monitoring the uterus, by a combination of rectal palpation and ultrasound, provides information on the response to treatment. Even if successfully treated and if the mare is to be bred, she must be considered a susceptible mare and managed accordingly.

In nonresponsive cases, hysterectomy can be performed after drainage of the exudate from the uterus, although great care has to be taken to prevent contamination of the peritoneal cavity.

Neoplasia

Uterine neoplasms are uncommon in mares. Leiomyomas, often referred to as fibroids, are benign mesenchymal neoplasms derived from smooth muscle and are often associated with the presence of fibrous tissue; leiomyoma is the most common neoplasm affecting the uterus of mares and, if small, does not necessarily cause reproductive failure. Leiomyosarcoma, lymphosarcomas, and adenocarcinoma are rare malignant neoplasms affecting mares.

Neoplasia affecting the equine uterus is usually discovered when palpation per rectum and transrectal ultrasonography are performed in broodmares during the breeding season. If the presence of a uterine neoplasm is suspected, uterine endoscopy should be performed and a biopsy of the tissue taken for final diagnosis. Surgical excision of a neoplasm is indicated when extensive haemorrhage and endometritis are present or when the presence of the neoplasm would be incompatible with establishing a pregnancy. Prognosis for future fertility is reduced, but pregnancy has been reported in mares with partial hysterectomy (Santschi & Slone 1994).

Uterine Tubes

The function of the uterine tubes and the tubal environment are essential for normal fertility. Mares are unique in that unfertilised oocytes are retained in the uterine tubes and not transported to the uterus. The mechanism(s) accounting for this phenomenon (selective transport of fertilised vs unfertilised oocytes) has not yet been fully elucidated but appears to involve secretion of PGE_2. A healthy uterine tube is responsive to embryonic signals, resulting in proper timing of tubal transport. Diagnosis of pathologies in the uterine tubes is difficult and often found only in postmortem examinations.

Postmortem examination of reproductive tracts found that salpingitis was not uncommon in mares; 37% had infundibulitis, 21% had ampullitis, and 9% had isthmitis (Vandeplassche & Henry 1977). In that study, 50% of mares were older than 15 years and 85 % were 11 years old or older. The infundibulum was generally found adhered to the uterus, mesovarium, or ovary. The incidence of adhesions on the right side was significantly higher than on the left side (Vandeplassche & Henry 1977). Although evidence of salpingitis could be detected in problem breeding mares, the incidence was no different from that noted in normal, fertile mares (Ball et al. 1997).

Uterotubal obstructions are less common in the mare than in the cow, although masses of collagen have been found in the oviducts of young maiden mares and pregnant mares and were observed more often in mares more than 7 years old (Liu et al. 1990). Historically, postmortem analysis of uterine tubes suggested that reduced tubal patency is not a major problem in mares. An examination of 700 postmortem specimens, primarily from mares over 11 years old, found that almost all oviducts were patent, although over 40% had adhesions involving the infundibula (Vandeplassche & Henry 1977). Only one uterine tube was found to be occluded when 1248 pairs of uterine tubes were flushed postmortem (David 1975, Vandeplassche & Henry 1977). Contrary to this, in a more recent report, Bennett and co-workers (2002) investigated 31 mares with histories of prolonged infertility for two to four breeding seasons.

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Evaluation of the reproductive tract using transrectal palpation, ultrasound, culture, cytology, biopsy, and hysteroscopy was performed and failed to provide a definitive diagnosis for the cause of infertility. The uterine tubes of these mares were assessed under general anaesthesia and demonstrated that 42% were bilaterally occluded and 19% were unilaterally occluded. Twelve mares that were bilaterally occluded had their uterine tubes flushed in an orthograde direction and were subsequently bred; a 67% conception rate was achieved. Recent work by Köllmann and co-workers (2011) demonstrated that the uterine tubes can be successfully observed and flushed using a laparoscopic flank approach.

In one author's practice (JRC), PGE gel is applied under laparoscopic guidance to the uterine tubes in mares suspected of having uterine tubal blockage. Allen et al (2006) demonstrated that laparoscopically guided administration of PGE_2 gel (dinoprostone) directly onto the surface of the uterine tubes was successful at restoring fertility in mares assumed to have occlusion, based on their reproductive histories. Fifteen mares that had failed to conceive over 1 to 4 years were treated, of which 93% conceived within the same or subsequent breeding season; 60% of mares conceived to their first mating (Allen et al. 2006). Application of PGE_2 to the uterine tubes is best performed during the breeding season (Martynski et al. 2015). More recently, uterine tube catheterisation has been described by Inoue (2013) via hysteroscopy and demonstrated that the technique was applicable to retrograde flushing of suspect blocked oviducts (Inoue 2017).

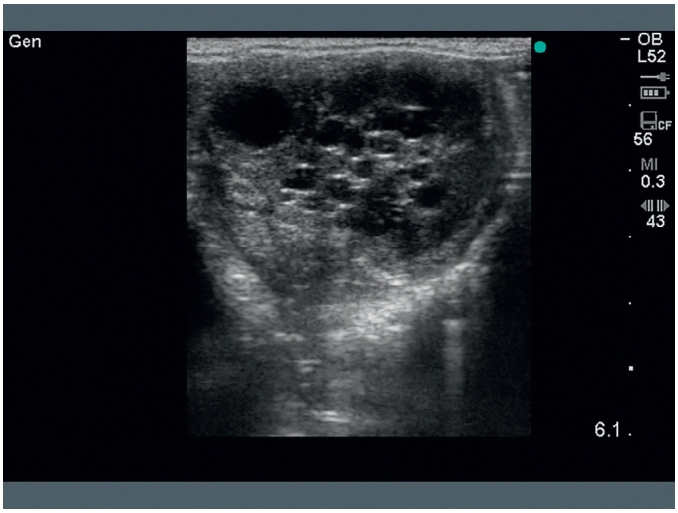
Most uterine tube abnormalities and periovarian structures diagnosed antemortem are remnants of embryological structures. Periovarian cysts, which are fairly common, are not endocrinologically active, do not usually interfere with the process of ovulation, and do not generally affect fertility. Occasionally, large cysts may be palpated or imaged with ultrasonography and may be confused with follicles. However, the lack of change in size or appearance of these structures is usually diagnostic.

Ovaries

The presence of an enlarged ovary in a mare may be normal or may be an indication of ovarian pathology. Consideration of the various reasons and careful diagnostic procedures are necessary to avoid surgical removal of normal ovaries. A thorough history, including changes in behaviour, oestrous cycle characteristics, sexual behaviour, and last observed oestrus, are important to consider. Ultrasonography, palpation, and hormonal assays are all helpful in arriving at an accurate diagnosis. In some mares, sequential examinations are beneficial in determining changes in the size of ovaries or various structures on an ovary. Various factors, such as season and pregnancy status, must be considered when interpreting a finding of ovarian enlargement. Large ovaries may be normal during the transitional periods in the spring and fall and are expected during early gestation.

Discovery of an enlarged ovary may be an incidental finding during a normal reproductive examination or may be stimulated by specific clinical signs. Behavioural changes or signs of colic in a mare warrant examination of the reproductive tract, with special attention given to the ovaries. Mares with a history of infertility or behavioural abnormalities are often suspected of having abnormalities of the ovaries and deserve a thorough examination before surgical removal of an enlarged ovary.

Cysts lying within the ovarian stroma near the ovulation fossa of the ovary arise from the surface epithelium and are often seen in older mares during examination of the ovary. They are known as 'retention', 'inclusion', or 'fossa' cysts and generally have no adverse effect upon fertility (Fig. 31.32). They are termed ovarian cysts by some authors, which may account for the diagnosis of 'cystic ovaries' in the mare. Sometimes 'cystic ovaries' are falsely diagnosed during the vernal transition, when a single follicle or multiple large ones persist without ovulating. Fossa cysts are normally only a few millimetres in diameter. Care must be taken not to confuse them with a normal ovary with several small follicles or even conceptuses. Careful examination allows accurate identification of their position. It is possible that sometimes, particularly if they are large and numerous as can occur in older mares, they could impede the release of the oocyte from the ovulation fossa. Small nodules, located within the loose connective tissue covering of the ovary (adrenocortical nodules), have also been identified.



• **Fig. 31.32** Ultrasound image of an old mare's ovary with numerous inclusion cysts.

Nonneoplastic Ovarian Enlargement

Anovulatory Follicles

During early gestation, ovarian enlargement is normal and should be expected. Ovarian enlargement is associated with increased follicular activity and subsequent ovulation (secondary corpora lutea) or anovulatory luteinisation (accessory corpora lutea) in response to the presence of eCG from the endometrial cups. An increase in follicular growth begins before day 20 of gestation. New CLs form around day 40 of gestation. Multiple CLs and haemorrhagic anovulatory follicles are commonly found from day 40 to 60 of gestation. Mares bred early in the season have greater follicular activity during the first 4 months of gestation than mares bred after July.

Anovulatory follicles are most commonly observed in mares near the end of the breeding season as they go into the autumn transition. Their significance is that the oocyte is not released but remains within the large unruptured haemorrhagic follicle. These follicles may grow to an unusually large size (70–100 mm) yet fail to ovulate. Instead, they fill with blood and develop a 'gelatinous consistency'. A thick wall (compared with a normal follicle) commonly forms. Ultrasonographically, free floating echogenic spots in the antrum of the follicle are observed, which increase in number as the follicle grows (Fig. 31.33). When the follicle stops growing, the contents become organised with an echogenic appearance and fibrin strands. They become firmer and then regress over time, usually disappearing within a month. Formation of luteal

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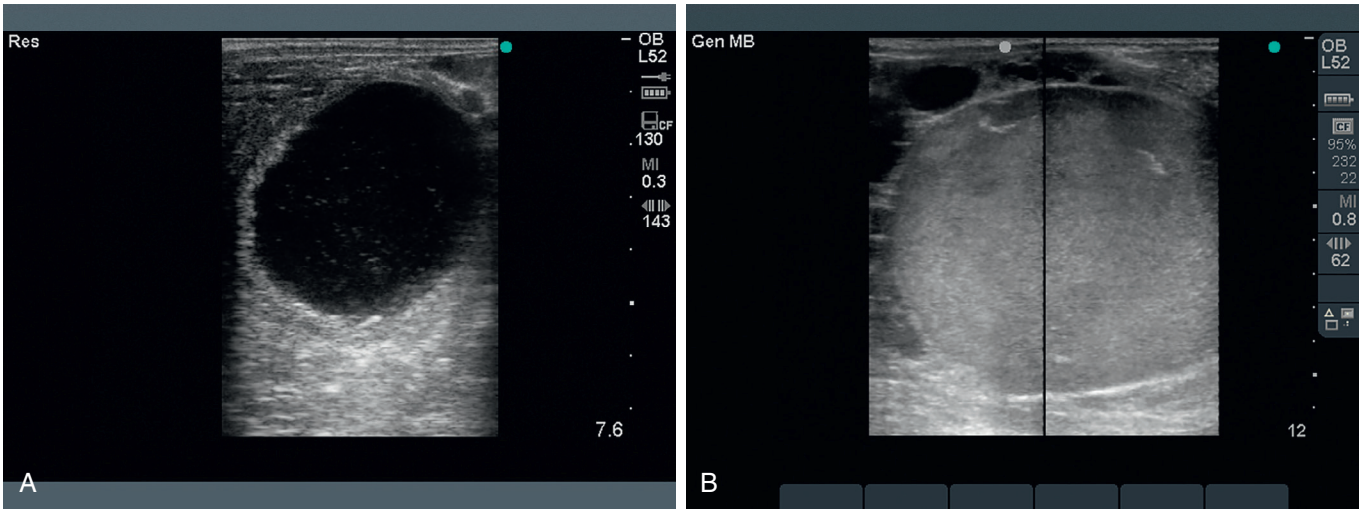
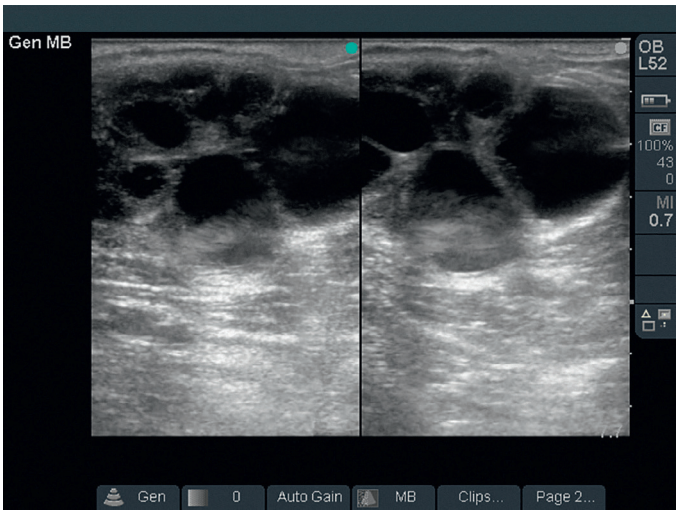
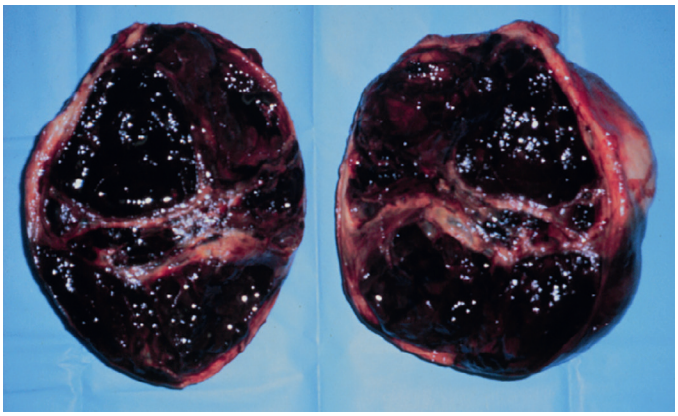


• **Fig. 31.33** (A) An early anovulatory follicle and (B) a large, more developed haemorrhagic anovulatory follicle.



• **Fig. 31.34** Transitional ovaries; note numerous follicles.



• **Fig. 31.35** Ovarian haematoma.

tissue around the periphery of the anovulatory follicle is usually minimal in a true 'autumnal follicle' that occurs at the end of the season. The cause of autumnal follicles is hypothesised to be due to changes in the hormonal status of the mare that occur with autumn transition.

During the spring, as mares undergo transition from winter anestrus to the normal ovulatory season, they undergo periods of prolonged anovulatory follicular development. These periods are characterised by the development of numerous follicles, at times quite large, on either or usually both ovaries. The follicles may persist for varying lengths of time and then regress and new follicles develop. This period lasts for a variable time depending on the individual mare, photoperiod, and other undetermined factors. The ovaries can be quite large during this time and may mistakenly be called cystic (Fig. 31.34). No treatment is necessary, although a combination of progesterone and oestrogen, as previously discussed, is often used to suppress follicular activity in an attempt to hasten the onset of ovulation and normal cyclicity. The transition period ends with the first ovulation of the season, after which follicular activity and ovarian size return to within normal limits.

Although there is no true menopause in mares, an age-related ovulation failure has been documented (Vanderwall et al. 1993). Some aged mares, particularly over 20 years, fail to ovulate despite showing oestrous behaviour.

An ovarian hematoma forms as haemorrhage occurs into the previous follicular lumen after ovulation. On occasion, this haemorrhage can be excessive, possibly due to an anticoagulant in follicular fluid. Hematomas can be very large, up to 20 cm in diameter or more (Fig. 31.35). Because this structure is a postovulatory phenomenon, the oocyte will have been released, and the mare may become pregnant if mated. They are able to produce progesterone, and there is no effect on the oestrous cycle. Although the ovulation fossa is still present, palpating it can be difficult if the hematoma is very large. The ultrasonographic appearance can vary, causing confusion with a granulosa cell tumour. Large fluid filled cavities may be observed, or it may have a more solid appearance, sometimes with fibrin strands. A trait that is at times useful in differentiating a hematoma from a granulosa cell tumour is the responsiveness to prostaglandin. Hematomas that are at least 5 or 6 days old usually respond to prostaglandin by decreasing in size because of the luteolytic effect, and the mare will return to oestrus. Granulosa cell tumours do not respond to prostaglandin treatment with any change in size, shape, or ultrasonographic appearance. With hematomas, because the follicle wall still undergoes luteinisation

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despite the presence of the hematoma, cyclicity remains unaltered; thus normal fertility is not compromised. However, the enlargement of the ovary due to the presence of a haematoma may persist for several oestrous cycles, even though the lifespan of the luteal tissue is normal.

The definition of an anovulatory follicle is one that fails to ovulate and thus fails to release its follicular fluid and oocyte. Two forms of anovulatory follicles exist. Approximately 15% appear to become 'cystic' and do not luteinise; these are sometimes referred to as persistent anovulatory follicles (PAFs). The other 85% consist of the follicles that luteinise without releasing the oocyte (McCue & Squires 2002). The most common form of luteinised anovulatory follicles is the haemorrhagic anovulatory follicle (HAF); far fewer show no evidence of bleeding and are defined as luteinised unruptured follicles (LUF). Anovulatory follicles can have a variety of morphological forms, and these can be functionally different. The haemorrhagic anovulatory follicle (HAF) is the most well recognised form because of its striking ultrasound characteristics.

The incidence of anovulatory follicles varies from 4.5% to 8.2% of oestrous cycles (Chopin et al. 2001, McCue & Squires 2002). The incidence of anovulatory follicles increases with age (4.4% incidence with mares 6–10 yrs, 13.1% incidence with mares 16–20 yrs; McCue & Squires 2002), and they are more likely to occur late in the season (Ginther et al. 2007, Cuervo-Arango & Newcombe 2010); some individuals appear to be more prone to the condition (McCue & Squires 2002, Ginther et al. 2006, Ginther et al. 2007, Cuervo-Arango & Newcombe 2010). In six mares that had a history of HAF in the previous season, the occurrence was 54% of all oestrous cycles (Ginther et al. 2006, Ginther et al. 2007). Cuervo-Arango and Newcombe (2010) estimated that the likelihood of mares having consecutive HAF was 31.5%; some mares may have a much higher incidence of HAF (25%) than other mares (Cuervo-Arango & Newcombe 2010). It should be noted that repeated HAF formation has been associated with persistent endometrial cups in mares with low levels of circulating eCG (Crabtree et al. 2011). As the definition of an anovulatory follicle is one that fails to ovulate, pregnancy rate is zero (McCue & Squires 2002).

The indicators of impending ovulation (decreased turgidity, loss of spherical shape, echoic specks in antrum, serration of granulosa, and an apical area) do not differentiate ovulatory from anovulatory follicles up until the day of expected ovulation (Ginther et al. 2006, Ginther et al. 2007). Follicles become haemorrhagic when blood appears to enter. However, in HAF, when ovulation would be expected if it was a normal follicle, it does not. Instead, the wall of the follicle becomes excessively serrated, and there is an increase in the echogenic particles in the follicle (see Fig. 31.33); sometimes they are so great that contents of the follicle appear like a swirling 'snow storm'. Often, there is an increase in size of the follicle at the same time (31.33b). Ginther et al (2006) and Ginther et al (2007) determined the Doppler blood flow characteristics of ovulatory and anovulatory follicles and demonstrated in anovulatory follicles an increase in the blood flow signal in the apical area (where the follicle ruptures via the ovulation fossa) in the 24 hours before expected ovulation; conversely, ovulatory follicles demonstrated minimal apical blood flow preceding ovulation. In the days afterwards, as the blood clot begins to organise, echoic bands or strands develop in the follicle, and these tend to 'quiver' when the follicle is ballotted (see Fig. 31.33B). This is due to the fact that follicular fluid contains a heparin-like substance slowing the clotting process (Stangroom & Weevers 1962). HAF appear to reach maximum diameter by approximately 3 days postovulation failure, increasing to as much as 70 to 100 mm, and become firmer. At the same time as this organisation of the HAF, the wall tends to become thicker as it becomes luteinised. The PAF physically mimics a dominant follicle and may persist for several weeks or months and then spontaneously regress. The LUF will demonstrate wall thickening as it undergoes luteinisation.

McCue and Squires (2002) reported that in over 151 HAF cycles, the average interovulatory interval was prolonged to 38.5 +/− 15 days. However Ginther and co-workers (2006) reported a normal interovulatory interval in seven HAF cycles, so it would appear that the response of the HAF to endogenous $PGF_{2\alpha}$ is variable, or that the normal luteolytic mechanism is not activated. In general terms the HAF and luteinised follicles will regress after treatment with $PGF_{2\alpha}$ but probably not at the same stage as a normal CL. This is because the vast majority of HAFs do luteinise, and thus a single injection of $PGF_{2\alpha}$ can be used to induce luteolysis once the HAF is functional with plasma P4 concentrations greater than 1 ng/mL or there is physical evidence of luteinisation (absence of floating echoic specks, thickened follicle wall). Typically, this occurs 5 to 7 days after initial signs of HAF formation. Another approach to prevent luteinisation and hasten structural regression of the HAF with return to oestrus is to administer serial injections of $PGF_{2\alpha}$ as soon as the formation of HAF is being suspected or diagnosed. This protocol was originally reported to prevent luteal formation in ovulating, cyclic mares, and it seems to be useful in promoting a fast resolution of HAFs. Daily or twice daily injections of dinoprost tromethamine (10 mg; IM) for 5 consecutive days are typically sufficient to prevent functional luteinisation and promote structural regression of the haemorrhagic/luteinising anovulatory follicle (Coffman et al. 2014). Nonluteinised anovulatory follicles do not appear to influence the oestrus and can be viewed as atretic structures with limited clinical significance.

The exact cause of HAFs is unknown; however, there have been some interesting developments in recent years. Cuervo-Arango and Domingo-Ortiz (2011) demonstrated that systemic treatment with a high dose of flunixin meglumine is able to induce HAF formation. In addition, Martínez-Bova and Cuervo-Arango (2016) demonstrated that intrafollicular treatment with $PGF_{2\alpha}$ inhibited this formation and restored normal ovulation, whereas systemic cloprostenol administration did not (Cuervo-Arango 2012). Studies of HAFs have demonstrated higher oestradiol concentrations a few days before HAF formation but no alterations in progesterone, LH, or FSH (Chopin et al. 2001, Ginther et al. 2006). Lower concentrations of inhibin and androstenedione have been demonstrated in the late follicular stage (Chopin et al. 2001).

The use of cloprostenol to 'short cycle' mares may induce some individuals to produce HAFs, and it appears to be dose dependent (Cuervo-Arango & Newcombe 2010). The premature stimulation of a young follicle with LH/hCG could also induce premature luteinisation (Coulam et al. 1982) without follicle rupture, as seen in pregnant mares under constant stimulation with eCG (Cuervo-Arango & Newcombe 2010). The use of prostaglandin can cause LH release (Jöchle et al. 1987), and this might be the mechanism for the effect of cloprostenol on HAF formation (Cuervo-Arango & Newcombe 2010). The general guidance in a mare prone to HAF formation is to use the lowest luteolytic dose of prostaglandin possible, such as 25 to 125 mcg cloprostenol (Cuervo-Arango & Newcombe 2010).

Ovarian Abscessation

Ovarian abscesses are often attributed to procedures involving puncture of the ovary, such as biopsy or follicle aspiration. As assisted

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reproductive techniques became more successful and therefore more popular, the incidence of ovarian abscesses was thought likely to increase. However, this has not proven to be the case. Moreover, not all ovarian abscesses can be attributed to iatrogenic causes. Ovarian abscesses have been reported in mares that have had no such procedures performed on them (Ramirez et al. 1998). In these cases, they are likely to be due to the hematogenous spread of bacteria, perhaps into a haemorrhagic structure, or may be associated with strongyle migration.

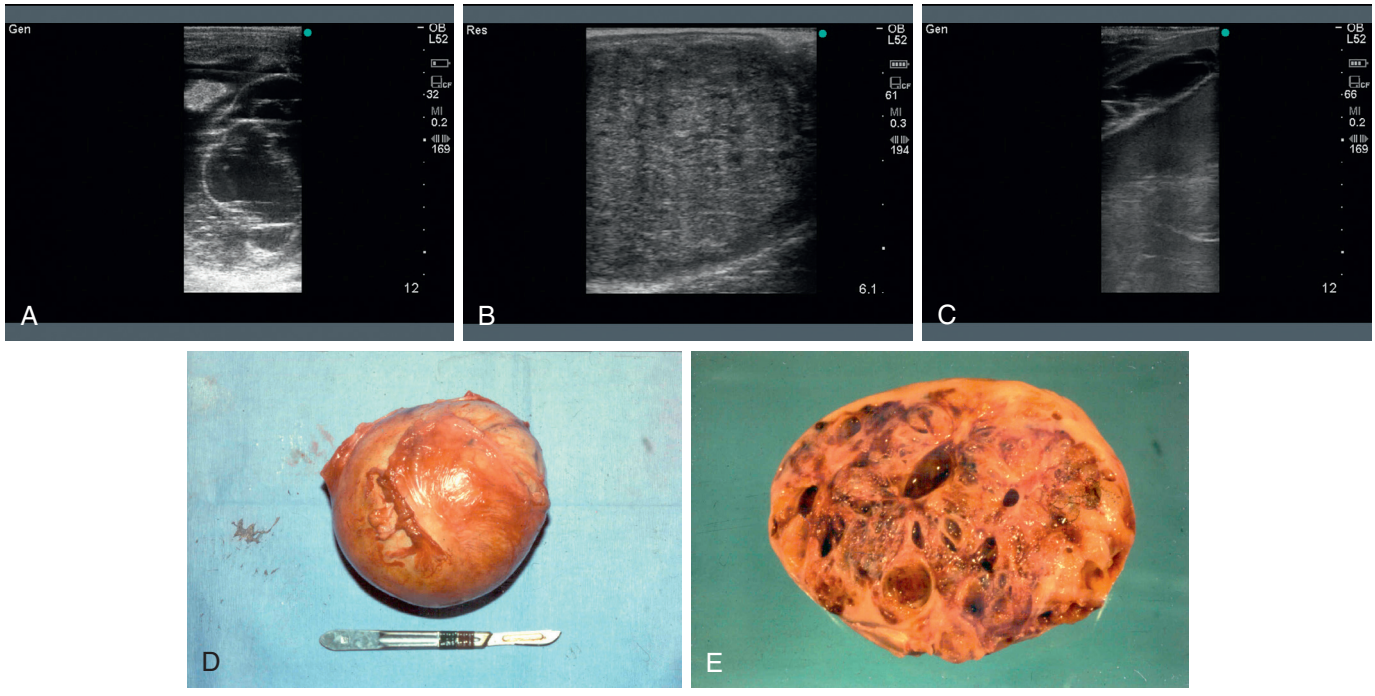
Affected mares may be febrile and anorexic with an elevated WBC. On ultrasonographic examination, the enlarged ovary typically has a thick-walled, fluid-filled structure; the fluid is usually hyperechoic. Medical management with long-term antibiotic therapy has been successful in treating these cases. Surgical removal of the affected ovary is an alternative treatment, but care must be taken that the abscess does not rupture in the abdominal cavity.

Ovarian Neoplasia

The granulosa cell tumour (GCT) is the most common ovarian tumour in the mare (McCue et al. 1998). They are either comprised primarily of granulosa cells or granulosa and theca cells, the latter being termed 'granulosa theca cell tumours' (GtCT) (Kennedy & Miller 1993). The tumour is predominantly slow growing and benign (Kennedy & Miller 1993). The mean age of affected mares is 10.6 years and ranges from 2 to 20 years (Meagher et al. 1977); rarely juvenile GCTs have been reported (Hultgren et al. 1987, Green et al. 1988, Charman & McKinnon 2007). GCTs have been detected in maiden, barren, pregnant and foaling mares (Stickle et al. 1975, Meagher et al. 1977, Crabtree et al. 2013).

Ultrasound examination of the affected ovary usually reveals an enlarged multicystic structure but can be in the form of a solid mass or single large fluid filled cyst (Crabtree 2011; Fig. 31.36A–C). They are usually unilateral and present with a small and inactive contralateral ovary (Meagher et al. 1977, Stabenfeldt et al. 1979). GCTs are hormonally active producing variable amounts of testosterone, oestradiol, and inhibin (Stabenfeldt et al. 1979, McCue et al. 2006). Mares demonstrate abnormal behaviour in the form of prolonged anoestrus, stallion-like behaviour, or persistent oestrus (Meagher et al. 1977, Stabenfeldt et al. 1979), depending on the predominant steroid hormone produced. Testosterone concentrations are elevated in approximately 50% to 60% of cases (McCue et al. 2006), and progesterone concentrations are almost invariably low, consistent with the absence of active luteal tissue (Stabenfeldt et al. 1979). Measurement of oestrogens is generally regarded to be of limited value as concentrations are variable and not always clearly related to the predominant behaviour expressed (Stabenfeldt et al. 1979). Inhibin is elevated in approximately 90% of cases (McCue 1992, Bailey et al. 2002), and it is hypothesised that inhibin produced by the GCT is responsible for the inactivity of the contralateral ovary through the suppression of pituitary FSH release (McCue 1992). GCT has, however, been described in mares that continued to cycle despite the presence of a GCT (Hinrichs et al. 1990, McCue et al. 1991, Nie & Momont 1992, Crabtree et al. 2013). It is assumed that these situations represent incipient tumours, which have not been present for long enough to inhibit activity in the contralateral ovary.

Due to the variabilities in the described hormones in cases of GCT, the normal variation in levels of inhibin during the oestrous cycle, and the difficulties encountered in interpreting GCTs in pregnant mares, serum anti-Müllerian hormone (AMH) has become the 'gold standard' in the diagnosis of GCT. Serum AMH concentrations are not different between normal cyclic mares and pregnant mares, and there are no significant variations in serum AMH concentrations during either the oestrous cycle or pregnancy (Almeida et al. 2011, Ball et al. 2013a). Elevations in serum concentrations of AMH appear to have a greater sensitivity than inhibin,



• **Fig. 31.36** Granulosa cell tumours with (A) a typical multiloculated appearance, (B) where there is a more solid state, and (C) associated with a large fluid filled structure. (D) Granulosa cell tumour after removal. (E) The tumour has been incised to show its multiloculated structure.

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testosterone, or the combination of inhibin and testosterone for the diagnosis of GCTs in mares, and elevations in serum AMH concentrations greater than 4 ng/mL seem to be diagnostic for equine GCTs (Ball et al. 2013a, Ball et al. 2014).

The differential diagnoses for ovarian enlargement in the mare include neoplasia, GCT, teratoma, serous cystadenoma, dysgerminoma, and thecoma (Crabtree 2011). Other tumours of the ovary are considered hormonally inactive, although cystadenomas with elevated testosterone levels have been reported (Hinrichs et al. 1989). Nonneoplastic differentials are a persistent anovulatory follicle, a haemorrhagic anovulatory follicle, ovarian haematoma, ovarian abscess, and ovarian enlargement during pregnancy (Crabtree 2011).

Medical complications due to or associated with the presence of GCT are uncommon; however, GCTs have been associated with colic, intraabdominal haemorrhage, adhesions to adjacent abdominal structures, and lameness (Meagher et al. 1977, Green et al. 1988, Crabtree 2011). Cases of malignant and metastasising GCTs have been reported (Meagher et al. 1977, Crabtree 2011), and this must be considered when advising mare owners regarding treatment. Single case reports also exist of cases of GCT associated with hypertrophic osteopathy, cutaneous lymphoma, granulomatous dermatitis, and secondary diabetes mellitus (Crabtree 2011).

Surgical removal of the affected ovary is recommended. Surgical approaches for tumour removal include colpotomy, flank and ventral midline laparotomy, and laparoscopy (Crabtree 2011). The selected surgical approach for ovariectomy is based on the size of the tumour, availability of surgical expertise, and economics. Standing laparoscopic ovariectomy is becoming the method of choice because it provides excellent observation of the surgical field ensuring haemostasis and reduced morbidity and hospitalisation time (Hubert et al. 2006). Larger ovaries can be drained or incised in order for them to be removed via a flank incision.

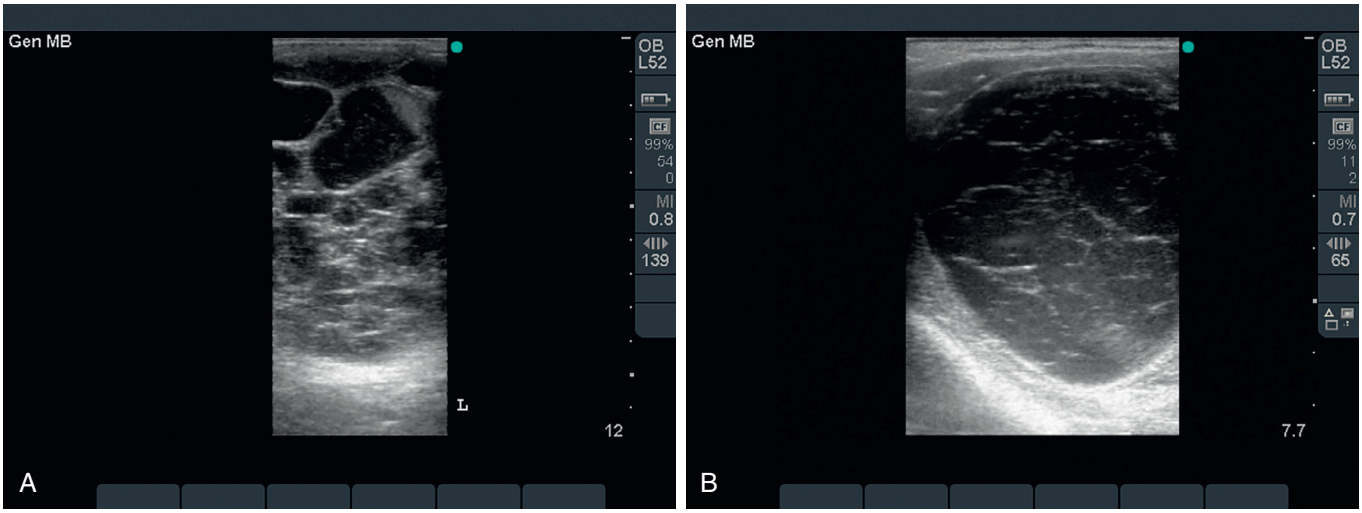
Testosterone and inhibin concentrations decline rapidly after surgical removal of the affected ovary (Stabenfeldt et al. 1979, McCue 1992, Bailey et al. 2002). Meagher and others (1977) reported that 74% of mares returned to oestrus, and 77% of mares known to have been bred postsurgery produced live foals. The time taken to return to cyclic activity is inevitably under seasonal influence, and even if the mare cycles within the same breeding season, she will most likely be bred the next season. The contralateral ovary returns to normal function on average 8.5 months after surgery in most mares (Meagher et al. 1977).

Behavioural changes alone can be misleading because many affected mares do not show any behavioural changes. Occasionally, owners express the opinion that their mare is difficult to handle or unmanageable when in oestrus and request veterinary treatment. Frequently, such mares are required to perform to a high level, and in the authors' opinion, most behavioural problems are not related to the reproductive system. If examination during a reported period of abnormal behaviour reveals marked follicular development, it is tempting to diagnose 'cystic ovaries' as the cause of the behavioural changes. On other occasions, the mare may even be in dioestrus when examined. In any case, owner pressure to perform an ovariectomy on suspicion of a GTCT should be resisted, at least until the mare has been monitored throughout several cycles to determine whether her behavioural problems are related to oestrus.

When it is thought that the behavioural problems are truly linked to oestrus, daily treatment with progesterone or a synthetic progestogen should prevent cyclical ovarian activity and oestrus. Although rare in an unbred mare, there is a possibility of an increased risk for endometritis in a mare on long-term progesterone supplementation, and she should be monitored for this. In addition, the problems may well recur after cessation of treatment. Another possibility is to breed the mare and establish pregnancy. Possible disadvantages of this approach are that she cannot be shown or compete in the later stages of pregnancy, and the problems may recur after birth of the foal. There may be some permanent conformational changes due to the pregnancy that could detract from the mare's showing potential.

The enlarged ovary may be smooth or 'knobbly', hard or soft, and may feel like there are multiple follicles present. Typically, the ovulation fossa on the enlarged ovary cannot be palpated, although with any greatly enlarged ovary, the fossa may be very difficult to palpate (see Fig. 31.36D and E).

Although ultrasonography is a useful adjunct, it may not yield a definitive diagnosis in many cases. The ultrasonographic image of granulosa cell tumours can be very similar to other ovarian abnormalities, especially haemorrhagic anovulatory follicles and haematomas (Fig. 31.37A and B). A variety of reported appearances



• **Fig. 31.37** Ultrasound image of (A) a granuloma cell tumour and (B) a haemorrhagic anovulatory follicle.

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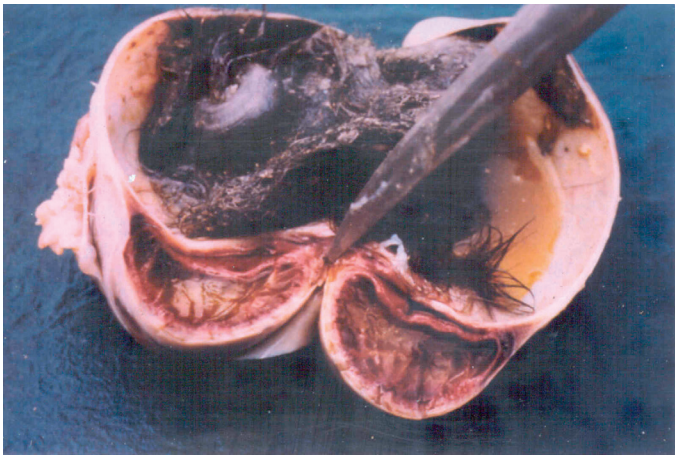

• **Fig. 31.38** Ovarian teratoma.

• **Fig. 31.39** Granulosa thecal cell tumour, which subsequently resulted in ovarian torsion.

makes diagnosis based solely on ultrasonography impossible in many instances.

Teratomas, although uncommon, are the second most common ovarian tumours; they contain at least two, if not all three, germinal layers. Most teratomas found in mares are benign. They usually contain hair and may also contain bone, teeth, and neural tissue (Fig. 31.38). Teratomas are usually an incidental finding because most are small and do not often cause significant ovarian enlargement. However, on occasion, large teratomas develop that result in ovarian enlargement. Teratomas do not affect the oestrous cycle and therefore lack obvious outward clinical signs.

Serous cystadenomas are neoplasms of epithelial origin usually found in an older mare; they do not metastasise. Although they have been found in mares with high plasma testosterone (Hinrichs et al. 1989), behavioural changes are not characteristic. The contralateral ovary is not affected, and it continues to have normal activity and does not atrophy; affected mares continue to cycle. On the affected ovary, the ovulation fossa is not obliterated and can be palpated.

Dysgerminomas are highly malignant tumours also of germ cell origin. They metastasise rapidly to the abdominal and thoracic cavity; they are considered to be the counterpart of the testicular seminoma. Because of their nature, they can affect other organ systems, and cases have been reported of associated hypertrophic, pulmonary osteoarthropathy (Vanderkolk et al. 1998). Clinical signs on presentation are therefore often unrelated to the reproductive system. These tumours carry a poor prognosis.

Ovarian Torsion

Ovarian torsion, a condition not uncommon in women, has been reported in a mare with a large granulosa–theca cell neoplasm showing signs of abdominal discomfort (Fig. 31.39) (Sedrish et al. 1997). Ovarian torsion may be suspected in mares with known ovarian enlargement if sudden signs of abdominal pain develop.

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32

Infertility in the Pig and the Control of Pig Herd Fertility

OLLI AARNO PELTONIEMI AND BAS KEMP

It is generally accepted that a sow should have expressed standing oestrus before 7 days after weaning her litter. If she fails to achieve this level of reproductive performance, then she can be classified as being subfertile or possibly infertile. In fact, sows that show oestrus early after weaning are known to be more fertile than those that show oestrus after a more prolonged interval (Fig. 32.1).

The owner or manager of a pig breeding unit will normally have set specific target values for the required reproductive performance of the herd. Together with the veterinarian responsible for the herd, there will be a policy on how to respond to a situation in which such a target is not being met. The remedy for the previous example, in which an individual sow has not expressed standing oestrus by 7 days after weaning or in which the average value for the herd is greater than the 7 days situation, may involve active intervention. The intervention may involve hormonal treatment, or increasing the time to allow for the individual(s) to recover from the 'metabolic burden' caused by the preceding lactation, or as a last result, culling of the animal. The choice of the remedy should be based, when possible, on the cause for the delay in expressing oestrus, its likely success, and the cost effectiveness. Advisors such as the herd veterinarian should provide the herd manager with agreed target values for reproductive performance, the measures that are needed to ensure that they are achieved, and the actions required to correct them. The most important targets for reproductive performance are defined in Table 32.1.

Methods for Examining the Reproductive System of the Subfertile/Infertile Gilt/Sow

Transrectal Palpation

The method is easy and fairly quick to perform and requires no equipment, although it is not applicable to gilts due to the small size of the pelvic canal which does not allow for insertion of the hand. In sows that have farrowed at least once, the method can be used with caution. After removal of faeces from the rectum, abundant amounts of lubricants should be used. If blood is observed on the operator's gloved hand at any time during the procedure due to damage of the rectal mucosa, then palpation should be stopped and the sow examined by alternative methods.

In diagnosing ovarian abnormalities, rectal palpation may be used to obtain an overall appreciation of the functional status of the ovary. It is possible to differentiate cystic ovaries from normal physiological ovaries based on their overall size as well as determination of the approximate the size of individual cysts. In most cases it is difficult to differentiate between normal size follicles close to ovulation and corpora lutea (CL) due to their similarity in size, their numbers, and texture. However, it may be possible to differentiate between small size follicles and normal physiological CLs, as the size of the former may be less than half of that of CLs. The obvious disadvantage of rectal palpation is that it can only be used in a proportion of breeding units because in most cases the feeding stall design does not allow for sows to be approached safely from behind.

Rectal palpation also needs substantial experience because the chances of false diagnosis, particularly discriminating between preovulatory follicles from CLs, is a risk.

Real Time Ultrasound

Real time B mode ultrasonography appears to be the most effective and accurate tool in diagnosing ovarian and uterine abnormalities in the gilt and sow (Kauffold et al. 2004a, Kauffold et al. 2004b, Kauffold & Althouse 2007). In transcutaneous examination of the ovary and the uterus, a 3,5 MHz sector probe is the optimal frequency; however, the widely used 5 MHz linear transducer probe will provide satisfactory images for clinical purposes. In rectal examination, the 5 MHz linear probe is very effective for diagnosis of ovarian disorders.

For transcutaneous examination, the probe is placed on the ventral abdominal wall just above the stifle joint and lateral to the mammary glands (Fig. 32.2). The probe is then directed towards the opposite tuber coxa and then redirected cranially until the urinary bladder is imaged. The ovaries and the uterus are located cranial and adjacent to the urinary bladder. Multiple sections of a single uterine horn may be seen in the same image due to the folding of the uterine horn that can be more than 1 m in length.

Ovarian function was examined in a study involving the use of transcutaneous ultrasonography of gilts and sows that had not conceived after breeding (Kauffold et al. 2004a, Kauffold et al. 2004b). The following is the frequency of ovarian structures identified: CL, 46%; follicles of 2 to 6 mm in diameter, 28%; periovulatory findings (follicles of 7–8 mm in diameter and corpora haemorrhagica), 19%; polycystic ovarian degeneration, 6%. These findings

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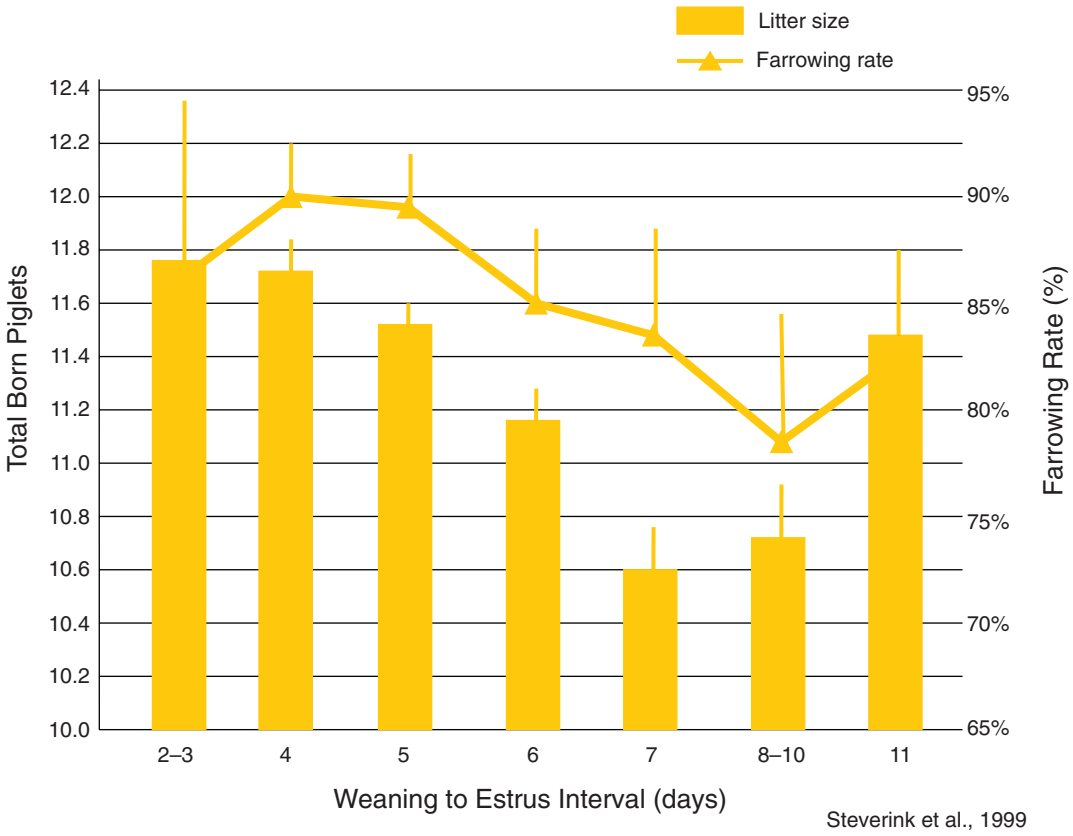
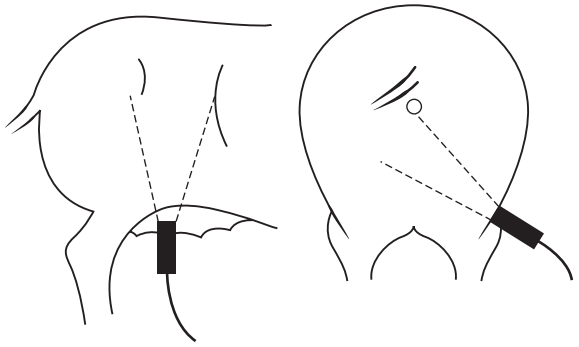


• **Fig. 32.1** Relationship between weaning to oestrus interval and fertility in the sow. (Data from Steverink et al. 1999.)



• **Fig. 32.2** Positioning of the transducer probe of the ultrasound scanner on the flank of the sow for transcutaneous scanning.

TABLE 32.1 The most important parameters regarding reproductive performance in a sow herd and their present target values

Parameter	Definition	Target
Weaning to oestrus interval	Days from weaning to oestrus	5 d
Farrowing rate	Proportion of mated sows that farrow	95%
Piglets weaned/ sow/year	Number of live piglets upon weaning the herd produces a year divided by the number of sows	30

indicate that, although the individuals had failed to conceive, the majority was undergoing normal cyclical ovarian activity, and as far as ovarian function is concerned, they have the reproductive potential to become pregnant if bred correctly.

The relative frequencies of ultrasound findings can vary from one farm to another, and assessing these frequencies may help to identify the problem on the farm and help in the employment of successful strategies. As an example, if ultrasound is used on Day 3 after weaning, it is very likely that an ovary with preovulatory follicles will be identified, as shown in Fig. 32.3A. However, if a high proportion of sows are found to have CL (see Fig. 32.2B) on their ovaries, then it indicates occurrence of oestrus during lactation in these sows. This would require the use of a different treatment than in sows in which ultrasound findings indicated the absence of CL sows on Day 3, but many present at Day 8 if they have not shown oestrus after weaning. In the latter, silent oestrus seems to have been the problem, and remedies should be to optimise oestrous detection strategies. Thus ultrasound examination after weaning may help to identify the problem and with advice given on the appropriate action.

Real time ultrasonography may also be used to determine the timing of the onset of puberty in gilts. The uterine horns increase in size and become readily identifiable with well-defined sections of uterine horns a few days before the onset of puberty (Martinat-Botté et al. 2003). This method was described as having over 90% sensitivity and specificity and is a useful diagnostic tool in herds with a problem of apparent delayed puberty or whether puberty has occurred but oestrus has not been identified.

The presence of embryos or fetuses can be reliably be determined from Day 25 after mating and onwards; before this stage, accuracy

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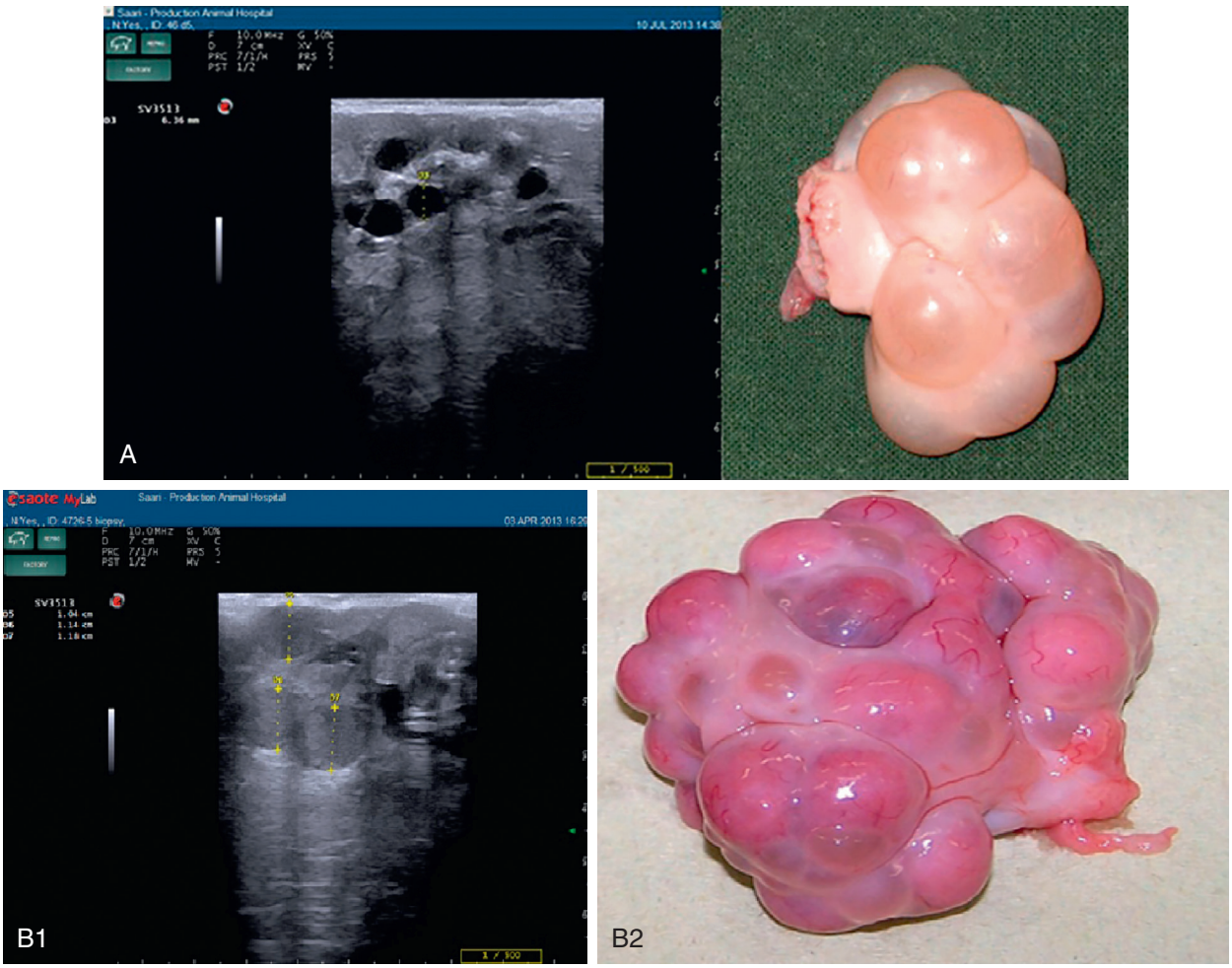

• **Fig. 32.3** (A) Ultrasound image *(left)* and photograph of an ovary with preovulatory follicles 6.3 mm in size. (B) Ultrasound image *(left)* and photograph of an ovary with functional corpora luteae 10.4 to 11.8 mm in size.

may not be good (Maes et al. 2006). A photograph and ultrasound image of a 35-day-old fetus are shown in Fig. 32.4A and B.

Vaginoscopy

The vaginoscope used for the examination of the vagina and cervix of the sow is usually 400 mm in length and 35 mm in diameter, which is considerably narrower than that used in the dairy cow. Vaginoscopy in the sow can be quite difficult to perform; it can be a very valuable tool to determine the source of a vulval discharge, which has been previously identified on the vulvar lips. The sources of discharge can be the urinary bladder, vagina, cervix, or uterus.

The sow or gilt should be adequately restrained, and the vulva are washed thoroughly and disinfected with an iodine-based disinfectant. The vaginoscope is inserted carefully using adequate amounts of lubricant; excessive quantities may compromise the evaluation of secretions. After assessing the volume and type of secretions, colour and appearance of the mucus membrane are evaluated. Furthermore, it is valuable to examine the caudal part of the cervix for the degree of patency of the opening (closed/half closed/dilated). Vaginoscopy is therefore helpful in determining the stage of oestrous cycle and differentiating between physiological and pathological.

Using Herd Management Programmes to Detect Reproductive Problems

Analysis of the records from herd management programmes is a very useful tool in diagnosing herd subfertility and problems associated with poor reproductive performance. However, basic epidemiological and statistical skills are needed when interpreting these data in order to find appropriate advice for their remedy and correction.

If there is a high proportion of older parity sows, which were inseminated at Days 14 to 18 after weaning, then it would indicate that an undetected lactation oestrus has occurred, and they are actually in the second heat. Furthermore, if the proportion of sows with weaning-to-oestrous intervals of between 25 and 28 days is high, then it may indicate that the first oestrus after weaning was actually missed.

Long intervals from weaning to oestrus in primiparous sows, and poor performance in their second litter may indicate problems in lactation management. However, herd management data needs to be interpreted with caution because the number of data available is often limited, and its quality is dependent on the accuracy of data collection and inserting into the database.

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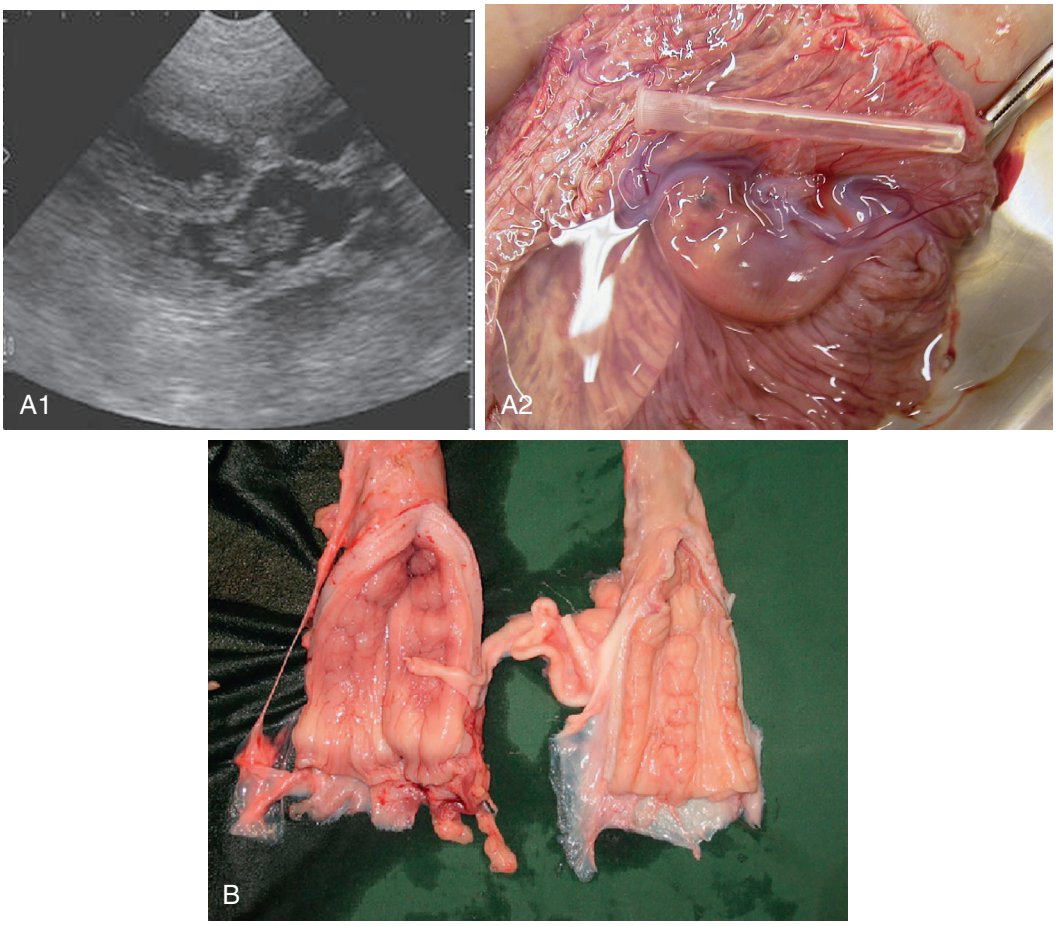

• **Fig. 32.4** (A) Real time ultrasonogram *(left)* and photographic image *(right)* of a 35 day old fetus. Organogenesis has begun as illustrated by the developing ribs and eyes. (B) Postmortem appearances of the cervix from two sows: the one on the left was in midoestrus and the one on the right was in the middioestrus at the time of euthanasia.

Postslaughter Examination of Female Genitals

A highly valuable tool for the accurate diagnosis of reproductive disease and disorders is postslaughter gross pathological examination. The genital organs, together with the urinary tract and bladder, are collected from specified sows on the slaughter line at the abattoir and submitted for pathological examination. To obtain representative samples, it is preferably to collect and examine as much of the material from as many of the problem sows as possible. In addition to a gross pathological examination, further analyses based on histological examination and bacteriological culture may be appropriate. However, the slaughter process may compromise the bacteriological findings, and the results should therefore be interpreted with caution. It is important that postslaughter examination is performed soon after sows are diagnosed as having reproductive problems. Long intervals between diagnosis and slaughter may mask the problems.

Microbiological Examination

Although considered by some to be of little value, the authors consider microbiological examination of swabs of a vulvar discharge to be a valuable method in determining the origin of the discharge, as well as providing useful information in relation to successful treatment of such cases (Oravainen et al. 2008). In order to collect samples, the vulvar lips are first washed three times with a polyvidone-iodine solution, rinsed with water, and wiped five times with gauzes saturated with chlorhexidine. The vaginoscope is then introduced into the vagina and a guarded swab inserted into the anterior vagina through the scope, and a sample can be collected. The swab is then placed in transport medium and submitted to the microbiological laboratory for culture.

Blood Sampling

In the search for pathogens and the measurement of reproductive hormone concentrations, blood sampling of gilts and sows is a basic clinical requirement. In modern housing environments (feeding stalls and a lying area), it often easier to approach a sow from behind when sows are closed into the feeding stalls. Therefore the tail vein (*vena coccygea)* or the saphenous vein (*vena saphena)* of the hind leg is often the first choice as a site for sampling. The tail vein runs longitudinally on the ventral midline of the tail, whereas the saphenous vein can be found on the caudomedial aspect of the hind leg and above the hock joint (Fig. 32.5). The tail vein is easier to access; however, it is a

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• **Fig. 32.5** Blood sampling of the sow using the saphenous vein of the hind leg.

more sensitive site and the sow will react to the insertion of the needle. The skin covering the saphenous vein is less sensitive, and blood sampling can often be performed without any reaction from the sow. However, a disadvantage of this site is that the vein may be difficult to find in fat individuals. Ear vein, jugular vein, and *vena cava caudalis* may also be used for blood sampling. However, using these sampling sites would involve restraining the sows, for example, by fastening them around the upper jaw with a rope snare and tying them up to a fence. In addition to the previously mentioned blood sampling methods, the saphenous vein of the front leg may be used in the gilt.

Causes of Subfertility/Infertility

Vulva Discharge Syndrome

Although the prevalence of vulvar discharge syndrome is generally considered to be quite low in the whole overall population of domestic sows, it may cause a serious problem in some individual herds (Oravainen et al. 2006b). The most important predisposing factor is thought to be the production environment in which the activity of sows is discouraged due to a too restricted activity area (< 2.5 m^2/sow space), inadequate bedding material, and restraining the sow in the feeding stall. In such circumstances, sows tend to drink less, resulting in reduced urine output. In addition, they also tend to lie down for prolonged period on dirty slats, resulting in heavy faecal contamination of the vulva and perivulvar area of the integument, so that there is an increased risk of environmental bacteria such as the E. coli entering the genital tract (Oravainen et al. 2006b).

The origin of the discharge may be: the uterus (metritis/endometritis); the cervix (cervicitis); the vagina (vaginitis); or the urinary tract. In the evaluation of the discharge, the normal physiology of the oestrous cycle should be kept in mind. During the luteal phase or pregnancy, the mucus membrane in the cranial vagina and caudal cervix should appear pale and dry, whereas during the follicular phase and oestrus, it should be more reddish in colour and moist. The cervix should be more or less closed during the luteal phase and pregnancy. During the follicular phase, the cervix may appear partially dilated which becomes more obvious during the oestrus. Physiological postweaning secretions associated with oestrus are usually clear, whereas the viscosity varies depending on the phase of the oestrous cycle. Secretions that are white, grey, or other coloured may be considered as pathological, especially if the volume is less than 5 mL and has an abnormal (unpleasant) odour.

It is useful to note the volume of the discharge. In urinary tract infections and metritis, the volume of the discharge is substantial (> 100 mL), whereas it is usually less in cases of vaginitis, cervicitis, or endometritis. In the interpretation of vaginoscopical findings, it is also useful to consider the association of discharge with the phase of the oestrous cycle. In endometritis, the discharge usually appears at the time of oestrus, whereas if there is a urinary tract infection, the discharge appears at the time of urination; blood and protein may be demonstrated, as well as a high pH value of 8 to 9 instead of the normal range of 4 to 5.

Mycotoxins

Mycotoxins, which contaminate pig food, can impair fertility as well as causing abnormal development of pig fetuses. They have been suspected as being the cause, not infrequently, of reproductive disorders in pigs. However, often it is quite difficult to link the actual mycotoxin contents found in a particular feed or bedding sample to the clinical signs observed because there are no guidelines for interpreting the significance of quantitative laboratory results.

Zearalenone is a classical oestrogenic mycotoxin that has been associated with clinical reproductive symptoms in pigs. Zearalenone has been reported to cause repeat breeding, signs of oestrus due to vulvovaginitis in prepubertal growing pigs, vaginal and rectal prolapses, pseudopregnancy, and signs of oestrus during pregnancy (Osweiler 2006). In field studies of clinical cases, although some of these symptoms were present, the authors have been unable to demonstrate significant zearalenone contamination of feed samples because the observed values were only slightly beyond the detection limit (Table 32.2). In a study by Zwierzchowski et al (2005) in which zearalenone was given orally at a dose of 200 microg/kg body weight, it caused disturbances in the process of development and maturation of ovarian follicles. Furthermore, although zearalenone contamination at levels of 1500 to 2000 µg/kg feed (1.5–2 ppm) has caused some signs of vulvovaginitis in prepubertal gilts, higher concentrations of 20 to 60 ppm are usually required for clinical signs to appear. Female pigs are more susceptible to zearalenone than adult males, who are relatively tolerant to the toxin in terms of reproductive function. However, young boars may experience reduced libido, preputial enlargement, and decreased testicular size if exposed (Osweiler 2006). Normal long-term reproductive function should return after removal of the toxin from the feed.

Beside zearalenone, the authors have observed significant deoksynivanole (DON) and nivalenole contamination in clinical samples. These Fusarium toxins may be of clinical significance (Table 32.2) because they have been reported to cause loss of appetite

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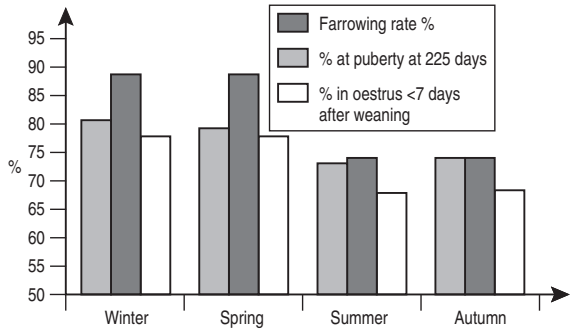
TABLE 32.2 Clinical feed samples from eight pig herds experiencing subfertility

Mycotoxin[a]	Range Found in Clinical Samples (μg/kg)	Detection Sensitivity (μg/kg)	Method Used
Zearalenone	2–3	2	fluid chromatography
Deoksynivanole (DON)	27–2256	10	gas chromatography – mass spectrometry
Nivalenole (NIV)	85–181	30	gas chromatography – mass spectrometry
HT-2-toxin	20–45	20	gas chromatography – mass spectrometry

[a]Analysis for the following toxins was also carried out, but they were not identified (detection sensitivity in brackets): ocratoxin A (OA, 1 μg/kg); 3-asetyldeoksi-nevalenole (3-AcDON, 10 μg/kg); Fusarenon-X (F-X, 10 μg/kg); diasetoksiskirpenole (DAS, 10 μg/kg); and T-toxin (20 μg/kg).
From: Production Animal Clinic of the University of Helsinki in 1999–2005.



• **Fig. 32.6** Major manifestations of seasonal infertility in the pig presented as percentages for each reproductive parameter. (Data from Peltoniemi & Virolainen 2006.)

and vomiting in pigs and thus may indirectly cause reproductive disorders because sows may lose body condition, thus impairing follicular growth. HT 2 toxins may be present in food prepared from Fusarium species–infected grain. Wet weather during the growing season and increased moisture during storage are risk factors for the presence of these mycotoxins in grain. Trichothecene contamination of grain, as an example, may therefore increase before harvesting.

Ergot alkaloids produced by the parasitical fungus *Claviceps purpurae* may affect rye, oats, and wheat. They are most commonly associated with agalactia in gilts and sows but may also cause low birth weight of piglets, low piglet survival, and low weight gain in neonatal piglets (Osweiler 2006). Abortion has not been associated with ergot toxins.

Seasonal Infertility

Seasonal infertility is defined as decreased fertility of the gilt and sow in summer and autumn. The signs of subfertility include: reduced farrowing rate, prolonged weaning-to-oestrous interval, and delayed puberty of gilts (Fig. 32.6). In feral wild pigs, decreasing day length in late summer and autumn would provide a physiological cue to indicate that it is not an optimum time for breeding because the piglets would be born in the midwinter. Thus in nature, that would most likely mean death of the newborn piglets and loss of the whole litter. The feral wild pigs are seasonally anoestrous, with the onset of the anoestrous period being under the control of the photoperiod (Tast et al. 2001). However, the end of the anoestrus period and return to cyclical ovarian activity are dependent on other environmental cues such as the increased availability of food (Mauget 1982). In the domesticated environment, cues such as increased availability of food and, in housed animals, a comfortable microclimate are absent, and these cues for seasonal breeding are removed. Therefore variable responses to decreasing daylight in the breeding activities of the female domestic pig population are frequently observed in late summer and autumn.

Seasonal infertility may be avoided by improving the reproductive management of the herd. This includes the use of a relevant light programme in which 16 hours of light and 8 hours of dark is provided in the breeding unit (see 'Light Programmes' later in this chapter). This recommendation is combined with advice to explore and, if needed, increase the light intensity to a level of at least 200 lux at the level of the eyes of the sow. Feeding of pregnant, group-housed females during late summer and early autumn is an issue of interest. Increasing feed availability in this time is an effective method of decreasing the stress of sows around the time of implantation of embryos and thereby reducing early embryonic loss and resultant return to oestrus. Moreover, stress of gilts and sows before insemination and during pregnancy is reduced by grouping them correctly according to size and age. Furthermore, other actions – such as genetic selection, reduced stocking density in summer to autumn, cooling systems applied especially during lactation in a hot production environment, and gonadotrophin treatments – may also be found useful in attempts to reduce seasonal effects on fertility (Peltoniemi & Virolainen 2006).

Cystic Ovarian Disease

Ovarian cysts are defined as persistent pathological fluid filled-structures (larger than 10 mm in diameter) present in the ovaries that prevent normal cyclical ovarian activity. Typically, ovarian cysts are multiple, each of which is 20 to 30 mm in diameter, so that the overall diameter of the ovary can be up to 100 mm, as shown in the real time ultrasound image in Fig. 32.7. B mode, real time ultrasound scanning, either transcutaneously or transrectally, enables the diagnosis of the disorder (Kauffold et al. 2004a). Recent findings in our laboratory indicate that the LH surge that triggers ovulation may not occur, and the follicles continue to grow beyond the normal size for ovulation of 7 to 9 mm in diameter. The impaired LH surge may be due to stress, zearalenone toxicity, hormonal (gonadotrophin) treatment administered at an inappropriate phase of the oestrous cycle (early dioestrus), or early induction of ovulation after parturition (e.g., early weaning protocols). The incidence of ovarian cysts has been reported to vary between 5% and 10%. Multiple ovarian cysts may contain some luteal tissue, which, if it persists, will inhibit subsequent

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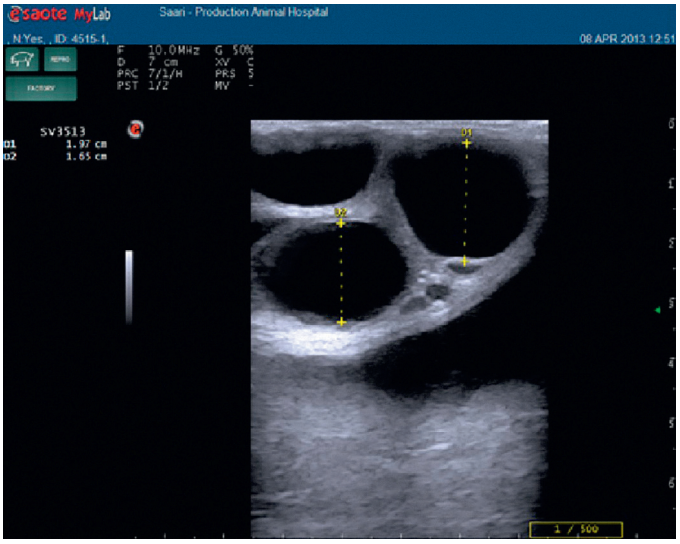
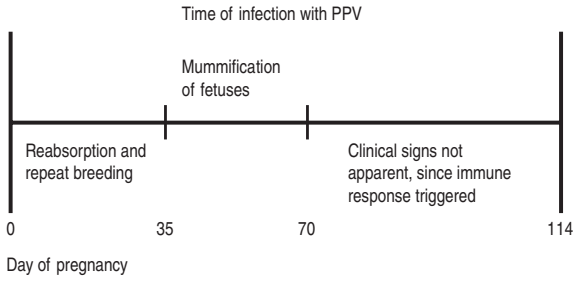


• **Fig. 32.7** A real time transcutaneous ultrasound image showing multiple ovarian cysts in a sow; the distance between the marks (+—-+) represents 10 mm.



• **Fig. 32.8** Temporal relationship between the development of immune competence of porcine conceptuses and the consequences of PPV infection throughout pregnancy in the gilt and sow.

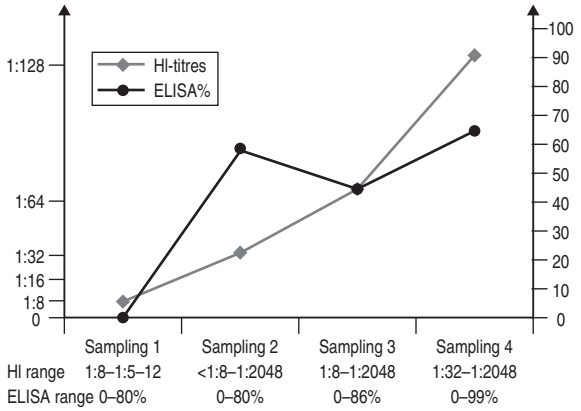
LH secretion and thus cyclical ovarian activity. Large multiple ovarian cysts generally severely compromise fertility; single and smaller ovarian cysts may increase the rate of repeat breeding and result in reduced litter size (Almond et al. 2006, Castagna et al. 2004). Early segregated weaning (weaning < 14 day of lactation) and a short weaning to oestrous interval (< 3 days) are known to increase the risk of ovarian cysts (Castagna et al. 2004). Prognosis and response to any treatment are generally poor, and culling of the sow is therefore often warranted.

Infectious Causes for Infertility

Viral Infections

Porcine Parvovirus

Porcine parvovirus (PPV) is a classical example of a viral disease that affects mainly the reproductive system. It has also been used as an example of the temporal relationship between the acquisition of immunocompetence and the development of the embryos and fetuses (Fig. 32.8). During the embryonic period of development, infection is likely to cause death and resorption of the embryos. In the fetal period, if the infection occurs before day 70 of pregnancy, the fetuses become mummified. However, if infected after immunocompetence has been acquired, the consequences are not serious, resulting in either slight weakness of the newborn piglets or the complete absence of clinical signs.



• **Fig. 32.9** HI titres and ELISA percentages of 39 gilts before and after vaccinations against porcine parvovirus. Sampling 1: before the first vaccination at the age of 6 months with the booster 3 weeks later (N = 39). Sampling 2: 2 to 3 weeks after the booster (N = 39). Sampling 3: in midpregnancy (N = 35). Sampling 4: after farrowing but before weaning (N = 13). (Oravainen et al. 2006a.)

PPV disease is widespread, with the herd prevalence in most countries exceeding 60 %. Thus for most producers, it is a disease with which they have to cope because eradication may not be a feasible exercise. The control programme involves vaccination of all breeding animals in the herd: gilts, sows, and boars. As PPV is known as the 'gilt disease', it is essential that gilts are vaccinated twice before they are inseminated for the first time. Vaccine-induced immunity is boosted in a stepwise manner, so that sows that have farrowed at least once clearly have higher antibody titres than unbred gilts (Fig. 32.9). Therefore in practice, signs of PPV may be seen in cases in which vaccination of replacement gilts has not been carried out or in which there is vaccination failure. Common vaccination failures can be due to the use of out of date vaccines, inappropriate injection site (neck instead of the base of the ear), storage problems (frozen/heated vaccine), and poor vaccination technique. It may also be that some animals do not have the required immune response because of a compromised physiological or immune status or a nutrient deficiency.

Circo Virus

Circo virus (PCV 2) infections are known to cause reproductive problems in sow herds in a number of countries, including South Korea and Spain (Chae 2005, Maldonado et al. 2005). The virus infects the conceptuses after transplacental transmission. Embryonic or fetal death, with the loss of the whole litter, may occur at any stage of pregnancy (Chae 2005). After embryo transfer, the majority of experimentally PCV 2–infected embryos did not survive for the 14-day period when there was observation of their development (Mateusen et al. 2007). However, in addition to abortion, an increased number of mummified fetuses, stillbirths, and weak but live-born piglets, may be observed. The oronasal route of transmission is considered to be the most common route of infection; horizontal transmission between pen mates and from one pen to another may be very efficient. However, transmission through semen may also occur, although it has not yet been proven to be a significant route (see review by Maes et al. 2008). Herds suffering from the epizootic phase of infection associated with reduced farrowing rates and increased stillborn rate may benefit from vaccination programmes that have recently been developed (Segales et al. 2007).

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Porcine Reproductive Respiratory Syndrome

Porcine reproductive respiratory syndrome (PRRS) is known to affect female pigs and cause respiratory disease in nursery and growing–finishing pigs, late term abortion, mummified fetuses, and weak but live-born piglets. However, transplacental infection may occasionally also occur earlier during pregnancy. Routes of shedding include saliva, nasal secretions, urine, and faeces. In semen the virus may be shed for a variable period of time ranging from 2 to 92 days after exposure (Christopher-Hennings et al. 2001). Sperm head defects, such as acrosome abnormalities and other morphological sperm abnormalities, may occur as a result of the infection (Feitsma et al. 1992). As a consequence of infection, there may be acute illness and infection of fetuses, resulting in abortion. It has also been reported that fetuses may die of hypoxia due to arteritis of the umbilical vessels (Lager & Halbur 1996). Exposure of PRRS-naïve replacement gilts to positively confirmed virus-infected tissues resulted in prebreeding infection of gilts, followed by the development of sufficient immunity to protect the subsequent pregnancy (Menard et al. 2007).

Aujzesky's Disease

A herpesviral disease of pigs, which mainly affects the reproductive system is Aujeszky's disease (pseudorabies). Herd prevalence of pseudorabies in Western countries is usually low, but once infected, a herd with continuous pig production may suffer from it for a long time. The route of transmission of the virus is usually by inhalation or ingestion. Replication usually occurs in the nasal and pharyngeal mucosa as well as in the genital tract. The virus may also be transmitted at coitus, and it has been detected in the urine, semen, and on the preputial lining after natural, as well as experimental, infection (Maes et al. 2008). Aujeszky's disease is characterised by nervous and respiratory clinical signs, together with pyrexia and often death of young piglets. Infection in adults may result in stillbirths and abortion. In adult boars and sows the clinical signs of the disease are seldom severe and usually consist of pyrexia, general depression, and anorexia, which last for up to a week. Of great significance to the breeding herd is the fact that the virus causes embryonic death, fetal mummification, and stillbirths.

Other Viral Agents

A variety of other viral infections have been associated with reproductive disorders in the pig (Almond et al. 2006, Maes et al. 2008). However, they are more likely to cause general symptoms such as high fever. These general symptoms have an indirect effect on reproductive function rather than a specific effect on the reproductive system.

Bacterial Infections

Leptospirosis

Leptospirosis is known to be a widespread disease compromising reproduction in the pig population all over the world. *Leptospira pomona* is the best known pig pathogen, but serovars *tarassovi, bratislava,* and *icterohaemorrhagiae* may also be pathogenical in pigs. Leptospires have a predilection for the kidneys where they persist and reproduce; they are then secreted via the urine into the environment intermittently for a period of up to 2 years. Adult, nonpregnant sows may act as asymptomatic carriers, and they may shed the bacteria in their urine to the environment for extended periods up to a year. Rodents are often thought to be the most significant reservoir species, maintaining the infection in the production environment. During the acute phase of the disease, mild general symptoms such as fever and anorexia may be seen; however, this phase often passes unrecognised. During the chronic phase of the infection, abortion rates of both gilts and sows increases considerably, especially during the second half of gestation. In the endemic the stillbirth rate may increase and newborn piglets may lack viability. Diagnosis is based on serological testing (microscopical agglutination test (MAT) and demonstrating the presence of leptospires in tissues and body fluids. Prevention is based on rodent control, good general hygiene management, and good urine drainage. High titres may indicate the presence of systemic disease, whereas low titres are evidence of venereal infection (Ellis 2015). Furthermore, antimicrobials (tetracycline and streptomycin) and vaccines are available for treatment and prevention.

Brucellosis

Brucellosis has been recognised as a disease associated with abortion in the pig since early 1900s; it is widespread in South America, Africa, and Asia. Although the bacteria may not be highly pathogenical for the pig, the zoonotic nature of the disease does present a risk to farm staff involved in the handling of pigs as well as interspecies transmission. The most frequently identified *Brucella* species found in the pig is *B. suis;* however, other species, such as *B. neotomae, B. ovis, B. canis, B. abortus,* and *B. Melitensis,* have also been identified. The most significant route of transmission is by direct contact between animals; infection is usually acquired through the oronasal or genital route. Sows may be infected when mated with infected boars (Lord et al. 1997). Adult pigs that are infected usually lack any general symptoms such as fever and loss of appetite. Abortion may occur at any stage of pregnancy. As sows usually eliminate the bacteria within 30 days after infection, a resting period lasting up to the length of two oestrous cycles may be enough, and subsequent AI may be warranted. Other signs of the disease include infertility, orchitis, posterior paralysis, and lameness. Boars may be unable to eliminate the bacteria and may act as carriers within a herd. Antimicrobial therapy is often ineffective, and eradication programmes may offer the best outcome.

Other Bacterial Diseases

Bacteria that trigger an acute phase response in the pig usually present a risk for reproductive health. For instance, endotoxemia caused by Gram-negative bacteria or general symptoms caused by *Erysipelothrix rhusiopathiae* can cause abortion at any stage of pregnancy. The better the immunity of the herd against these pathogens, the lower the risk to reproductive health.

Reproductive Management to Ensure Optimum Fertility

Management of Lactation

An important way to ensure optimum fertility and thus reduce the level of subfertility is by preventing excessive weight loss during lactation. Generally, some weight loss is both inevitable and acceptable, but weight losses during lactation (measured from after birth of the piglets to weaning) should be less than 12.5%. Optimising feeding and nutrition during lactation can achieve this outcome, as will be discussed in this chapter in the section 'Feeding the Sow'.

Another method of relieving the sow from the 'metabolic burden' of lactation is by reducing the effects of the suckling stimulus. Reducing the number of piglets during the whole or part of lactation may reduce milk production and may also reduce the inhibition of the suckling- induced suppression of LH release

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by endogenous opioids. Low numbers of piglets during lactation have been shown to improve reproductive performance of sows, but the reduction in the number of piglets has to be substantial to have any effect, partly because piglets in smaller litters will consume more milk per piglet.

Effects of interrupted suckling (a daily temporary removal of the whole litter) or split weaning (a permanent removal of part of the litter a few days before completing weaning) on subsequent reproduction have been reviewed by Matte et al (1992). In general, effects of such techniques on the weaning-to-oestrous interval are variable, and relatively small. Recent studies from our laboratory and in Australia (see Kemp & Soede 2012, for review) show that intermitted suckling can induce lactation oestrus, especially when intermitted sucking starts around the normal weaning age and is combined with boar stimulation. Oestrus can be induced during lactation in about 70% to 90% of the sows within 6 days after start of induction; farrowing rates and litter size are comparable to controls. Success is dependent on parity (primiparous sows do not respond very well) and breed. Lactation oestrus has the potential advantage that the lactation period can be extended while sows are pregnant to allow piglets to be better developed when they are eventually weaned. However, lactational oestrus may also be considered disadvantageous because the signs are often poorly expressed by the sow when there is inadequate boar stimulus; it is also laborious, and not all sows will respond to intermitted suckling and boar contact, which results in reduced synchrony in planning process.

Generally, sows that lose more than 12.5 % of body weight during lactation have an increased risk of lower subsequent farrowing rates and litter size; first litter sows are at greater risk. An approach that allows the first litter sow to recover from the effects of the previous lactation is to inseminate or serve her at the second oestrus after weaning instead of the first one ('skip a heat'). Missing the first oestrus can improve pregnancy rates by 15% and subsequent litter sizes by 1.3 to 2.5 piglets (Clowes et al. 1994, Werlang et al. 2011). When using this approach, an intense oestrous detection regime is important to ensure that the second oestrus is observed.

Whether or not these techniques should be used is a matter of economic calculations. The costs of an extended weaning-to-service interval should be balanced against the benefits of improved pregnancy rates and litter sizes.

Gilt Management and Feeding

Gilt development has been an important aspect of optimising litter size. Generally speaking, mating gilts at a young age or at a relatively low body weight has no negative effect on first litter size. However, second litter size can be compromised because young sows still have the impetus to grow while feed intake is limited during the first lactation. Sows with low weaning weights after the first lactation may have a second litter that is smaller in number than the first litter. This phenomenon is known as the 'second litter syndrome'. This has persuaded many veterinarians in practice to recommend inseminating gilts when they are older and are more mature. First breeding ages of 250 to 260 days are nowadays common in Europe, and this may be too old from an economical perspective.

Sows that become fat during pregnancy have a lower voluntary feed intake during lactation. In addition, insufficient feed intake during pregnancy results in thin sows, which cannot be compensated for by increased voluntary feed intake during the subsequent lactation. Thus these thin sows may have a prolonged weaning-to-oestrous interval, lower subsequent pregnancy rates, and smaller litter sizes. Therefore gilts should be fed according to their requirements for maintenance, reproduction, and growth but not be overfed. In practical terms, gilts may have an energy intake of 24.8 and 36.1 MJ metabolisable energy (ME) per day at the beginning and end of pregnancy, respectively (Everts et al. 1994).

Feeding the Sow

During lactation, energy requirements of sows are estimated to be 12.6 MJ ME per 100 kg of sow weight for maintenance, plus 6.2 ME per suckling piglet for milk production. For a 200 kg sow nursing 12 piglets, this means a daily ME intake of 99.5 MJ ME per day. In most commercial sow diets, this represents a daily feed intake of 7.7 kg/day. Due to low parity or high farrowing house temperatures, this daily requirement is sometimes not met. Thus many sows should be fed to appetite during lactation. Only in breeds with an innate high appetite is it advisable not to exceed recommendations because lactational oestrus may occur.

It has also been shown that the last week of lactation is a critical period as gonadotrophins begin to stimulate follicular growth sometime before weaning. As far as follicular growth is concerned, energy balance and feed intake of the sow during early lactation is secondary to that in late lactation. If feed is restricted during the last week before weaning, the weaning-to-oestrous interval is extended, the ovulation rate is decreased, and embryonic survival is reduced (Table 32.3) (Zak et al. 1997a). Sows that were deprived of food during the last week of lactation also had a lower number of large follicles, and the *in vitro* maturation capacity of ova from these follicles proved to be reduced (Zak et al. 1997b). In addition, the quality of the fluid in these follicles was poorer because slaughterhouse-derived ova matured *in vitro* in the follicular fluid from these sows showed impaired maturation.

These data suggest that poor nutrition during lactation impaired follicular development during and after lactation, resulting in a lower ovulation rate and an impaired quality of ova and follicular fluid. This may also explain increased embryonic mortality during the subsequent pregnancy. A lower ovulation rate and increased embryonic mortality may both result in a lower litter size at farrowing. When the ovulation rate is low or embryonic mortality is high, the number of viable embryos may be below the minimum of two per uterine horn.

TABLE 32.3 Weaning-to-oestrus interval (WEI), ovulation rate (OR), and embryo survival rate (ESR) at day 28 of pregnancy of sows with different pattern of feed intake during a 28 day lactation period

	Feeding Pattern		
	AA	AR	RA
WEI (h)	88.7[a]	122.3[a]	134.7[a]
OR	19.9[a]	15.4[a]	15.4[a]
ESR	87.5[a]	64.4[a]	86.5[a]

AA = sows receiving ad lib feeding; AR = Ad lib the first 21 days, 50% of ad lib the last 7 days; RA = 50% of ad lib the first 21 days, ad lib the last 7 days.
[a]Significant difference.
From: Zak et al. 1997a.

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In that case, sows will not remain pregnant and will return to oestrus after 21 days.

Some reports show that feeding lactating sows more than twice a day will improve overall feed intake compared with ad lib feeding systems. A 10% higher feed intake has been observed when using self-feeders instead of feeding twice a day. Generally, it is advisable to remove feed from the trough once daily to prevent it from becoming mouldy and sour.

A gradual increase in feed intake during the first week of lactation and a good appetite thereafter, without periods of loss of appetite, have been found to be a superior pattern of feed intake (Koketsu et al. 1996b, Koketsu et al. 1996a). The sows in this study had the best reproductive performance in the 'follow-up' period. If sows are overfed at the onset of lactation, sometimes there is a drop in feed intake during the remainder of the lactation. In these sows, reproductive performance is lower. Therefore it is advisable to increase feed intake gradually from the first days of lactation. Two kg of diet at farrowing and a stepwise increase of feed intake of 0.5 kg per day until the recommended feed intake is reached has been recommended as a feeding strategy during the puerperium.

Use of the Boar

The use of the boar for oestrous stimulation and detection of standing oestrus is essential in the modern reproductive management of the sow. Proper expression of oestrus signs and therefore adequate detection of oestrus require a direct nose-to-nose contact between sows and the boar. The best management routine is to allow a direct nose-to -nose contact with the boar at least twice a day while applying the back pressure test on the sow. This contact should be allowed daily after weaning until AI has been performed and standing oestrus has ceased in the sow. Introducing the boar to the sow in order to detect oestrus is better than the continuous housing of the boar and sows adjacent to each other. Daily nose-to-nose contact between gilts younger than 5 months of age and the boar has been shown to enhance puberty, and this daily routine should be carried out until the gilts have been successfully bred.

Timing of AI

Timing of AI is an important factor in ensuring good fertility. Inseminating too early may be unsuccessful, whereas if the sow is bred too late after ovulation, endometritis, resulting in decreased litter size, may occur. In the sow, standing oestrus lasts for about 48 hours on average, and ovulation takes place when two-thirds of the duration of standing oestrus has passed. The duration of ovulation (from the first ovulation to the last) is about 2 to 4 hours. Sperm may remain viable in the female genital tract for 20 to 24 hours, and ova may be fertilised for up to 2 to 4 hours after ovulation. Therefore an optimal time for AI has been found to be 0 to 24 hours before ovulation. In practice, timing of ovulation is not usually monitored, and a second insemination 12 to 24 hours after the first AI is warranted. Artificial insemination after ovulation and at the end of standing oestrus should be avoided in the event that endometritis has been caused by the late AI. Reduced fertility may be caused by inadequate oestrous detection routines or because first inseminations are timed too late in oestrus. The latter can be a problem in sows with slightly prolonged weaning-to-oestrous intervals and those that have a short duration of oestrus, in which case they should be inseminated early after the onset of heat. In some farms the sow population as a whole has an average short duration of standing oestrus. To verify this, the farmer should be advised to have the ovulation time of his sows verified by the herd veterinarian or a specialist veterinarian. Insemination routines can be adopted to improve the timing of the first insemination.

Light Programmes

The light intensity in the breeding area should be at least 200 lux, and light cycles provided for the sows should be consistent. Continuous light for 16 hours, followed by 8 hours of darkness, is a good rotational regimen to optimise fertility. In the rotational regimen, 8 hours of light and 16 hours of darkness should be used in the lactation unit, followed by 16 hours of light and 8 hours of darkness in the breeding unit. The most important point regarding the use of lighting regimens is that they should be consistent, with a clear difference between night and day, so that the sow can adapt her physiological functions accordingly (Tast et al. 2005).

Hormonal Treatment Protocols

Synchronising Oestrus in Gilts

A group of gilts may be synchronised for oestrus using physiological and pharmacological approaches. The physiological approach involves regrouping of gilts and placing them adjacent to a boar (Pearce 1992).

If a larger scale, a more precise synchronisation is required, so a pharmacological approach may be useful. A synthetic progestagen given daily by mouth for 14 to 18 days may synchronise oestrus and hence the time of breeding of gilts; standing oestrus is likely to occur 4 to 6 days after the last day of progestagen administration. Furthermore, gonadotrophins given parentally, 1 to 2 days after the last day of progestagen treatment, enhanced follicular development and ovulation. Results from Soede et al (2007) showed that gilts synchronised with progestagens had improved follicular development when under the influence of the progestagen, resulting in higher ovulation rates after their withdrawal.

After Weaning

Use of Gonadotropins

The use of hCG + eCG directly after weaning may result in an improvement of the weaning-to-oestrous interval, but it may also result in lower pregnancy rates or lower litter size. The latter may be related to differences in follicle development at the time of hCG + eCG injection, but no data are available to substantiate this.

Use of Progestagens

Epidemiological studies have shown that a short weaning-to-oestrous interval is a risk factor for the 'second litter syndrome'. Sows with a long weaning-to-oestrous interval are more likely to produce a larger second litter (Morrow et al. 1992). This may provide an explanation as to why ovulation rates or embryo survival may be improved by allowing the sow to recover for a longer period after lactation. For such a recovery, sows may be given resting period for the duration of a whole oestrous cycle ('skip a heat'). Alternatively, sows may be treated with a progesterone analogue, such as altrenogest, after weaning to artificially extend the weaning-to-oestrous interval. Treatment periods between 3 and 7 days after weaning have been used but have resulted both with positive and negative effects on the subsequent farrowing rate and litter size. However, daily treatment 14 days after weaning has resulted in sustained reproductive benefits (Van Leeuwen et al. 2011). The positive effects on ovulation rate, embryo development,

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and embryo mortality can be explained by restoration of follicular development under progestagen treatment after lactation.

Control Programmes for Oestrus and Ovulation

Traditionally, good fertility at a herd level is based upon the weaning of sows after 4 weeks of lactation, after which oestrus is detected, and is followed by double artificial insemination on the first and second day of standing oestrus. Therefore the timing of AI is based on the time of beginning of observation of standing oestrus. The optimal time for AI is within the last 24 hours before ovulation. Real time ultrasonography can be used to assess if sows are correctly inseminated relative to ovulation because follicle development is even measurable on the ultrasound image (see Fig. 32.4A). At ovulation, the follicles become filled with blood, and the hypoechogenic follicles cannot be seen any more. Insemination after ovulation is to be avoided.

The use of exogenous hormones provides the farmer with further possibilities for the timing of insemination close to the time of ovulations, so that less time is spent on oestrous detection. Progestogens, such as altrenogest, are used to time the onset of the follicular phase. eCG is used to stimulate and synchronise follicular development during the follicular phase, and GnRH analogues, hCG, and porcine luteinising hormone may be used to induce ovulation. Use of one or more of these hormones may help to reduce the time and effort spent on oestrous detection. The sequential use of these hormones may be used to induce ovulation, so that and AI may be performed at a fixed time after the administration of the hormones (fixed-timed artificial insemination, FTAI) without the need to observe behavioural oestrus. In gilts, results of timing of AI based on oestrous behaviour after synchronisation as described previously result in pregnancy rates of about 70%. In sows, in addition to the protocols described previously, successful results have been reported by eCG treatment at weaning, followed by GnRH or pLH at the time of the onset of oestrus, and either single or double AI thereafter.

In addition, single or double FTAI may be applied after a protocol based on altrenogest as described previously, followed by eCG and then GnRH or pLH a few hours later. Furthermore, FTAI may be carried out after the use of a protocol of eCG at weaning, followed by hCG, GnRH or pLH, and then FTAI. Studies of the results from these FTAI programmes have reported similar results to the traditional use of AI, based on the time of detection of standing oestrus (De Rensis & Kirkwood 2016). A relatively new approach is the use of a single dose of a GnRH analogue, some 90 to 110 hours after weaning, to induce and synchronise ovulation.

Veterinary Herd Fertility Control Programmes

The implementation of herd fertility control programmes based on the advice and support of the herd veterinarian may prove to be very useful for the pig farmer. This can be combined with other herd health work during regular herd visits. The programme can be based on the creation of specific targets for fertility related to the particular herd. For example, the targets may include: frequency and success of farrowing assistance and piglet mortality; proportion of sows in oestrus after weaning; accuracy of AI in relation to behavioural oestrus and the time of ovulation; pregnancy rates; abortion rates; number of piglets born and weaned; and number of stillborn piglets.

Regarding oestrous detection, the timing of AI in relation to ovulations can be assessed as previously described, and thus the arrangement of AI in relation to oestrous detection can be organised more precisely. Sows not 'coming into oestrus' within the week after weaning may be subjected to a gynaecological examination approximately 1 week after the expected oestrus; this will include palpation of the cervix and ultrasound scanning of the ovaries.

Sows returning to oestrus after AI (repeat breeders) can be examined during the visit as well. Pregnancy detection at 4 weeks, followed by a subsequent detection at 7 weeks to account for early pregnancy loss, is generally recommended and frequently done by the farmer or herdsperson on a routine basis. However, when there are fertility issues, the use of advanced ultrasound technology provides opportunities for early pregnancy detection at 17 days after insemination and may be utilised by the herd veterinarian in such situations. Cases where routine pregnancy checking results in an inconclusive outcome, can be further examined by the herd veterinarian during the following visit.

The frequency of veterinary fertility control visits will depend on the schedule of events in the batch farrowing system of the herd. In large herds in which large groups of sows are weaned every week, weekly visits will be warranted. In smaller herds in which the weaning groups of sows occur every second week, third week, or after more weeks, the frequency of scheduled visits can be reduced and may be set accordingly.

Antimicrobial Treatments

In uterine infections, the causative bacteria may originate from the environment, or they can be transmitted between animals – for example, via natural mating or AI. In postpartum dysgalactia syndrome (PDS) and in postmating endometritis, the bacteria causing the disease are usually of environmental origin; coliforms have been reported as the most common contaminant isolated at culture (Klopfenstein et al. 2006, Oravainen et al. 2006b). In the vaginal discharge syndrome, between-animal transmission may be important, and *Actinobaculum suis* has been reported to be a likely pathogen. For coliforms, in addition to supportive therapy (fluids, NSAIDs, and oxytoxin), antimicrobials such as ampicillin and trimethoprim-sulfa may be indicated. For *Actinobaculum suis,* procaine penicillin G may be the antimicrobial agent of first choice (Friendship 2006, Peltoniemi et al. 2003). In the treatment and prevention of leptospirosis, tetracycline and streptomycin may be indicated (Friendship 2006).

Genetics

It appears that various physiological changes may have occurred in the reproductive function of sows over recent decades (1954–2006). Although ovulation rates have nearly doubled from 13 to 16 to 20 to 26, embryo survival rate has decreased from 75% to 79% to 60%, and fetal survival has decreased from 69% to 74% to 50%. The increased ovulation rate is apparently due to selection for larger litter size, and good fertility is generally expected if the sow is correctly inseminated; the fertilisation rate is generally good (85%–95%) in the sow. Uterine capacity may be the most significant factor that has reduced the rate of embryonic survival because experiments simulating uterine crowding have shown that reduced uterine space increased embryonic and fetal mortality and reduced fetal growth (Pere et al. 1997). In the future, as litter size may continue to rise due to increasing ovulation rates, negative effects on birth weights of piglets resulting in a compromised start to neonatal life is anticipated. Gilts that show good oestrous signs at puberty may show better signs of oestrus after weaning, and thus

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the intensity of oestrous signs may be used as a selection criterion for replacement females in the future (Sterning et al. 1990).

Another aspect that has changed in the contemporary sow is their reactivity to low feeding levels during lactation. In the literature published during the 1970s and early 1980s, feed restriction resulted in quite dramatic effects on the weaning-to-oestrous interval (about 10 days) but with hardly any effects on ovulation rate and embryo survival. In more recent papers, the effects of feed restriction during lactation on weaning-to-oestrous interval are quite small (less than a day), whereas effects on ovulation rates and especially on embryo survival are far more pronounced. Feed restriction seems to decrease ovulation rates by about 2 to 4, and embryo survival by about 10% to 20% in modern sows. This change in responsiveness of sows due to feed restriction during lactation is a result of a strong selection against weaning-to-oestrous interval in the past 20 years. This implies that, in the modern sow, effects of low feed intake during lactation on weaning-to-oestrous intervals is less pronounced but that effects on litter size are more pronounced. The first litter sow is most vulnerable due to a restricted feed intake capacity. Based on the analyses of 135 United States farms, Morrow et al (1992) showed that, in about 40% of the farms, the second litter size of their sows was lower than or equal to the first litter produced. Since then, this phenomenon has been known as the 'second litter syndrome' (Morrow et al. 1992).

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33

Infertility in the Bitch and Queen

GARY C. W. ENGLAND

The dog and cat are among the most common species kept as pets. There have been considerable advances in our knowledge of canine and feline reproduction, and over the last few years, there has been increased awareness of infertility in these species. In the dog in particular, this has been the result of attempts at selective breeding for working and show purposes, for assistance animals for disabled people, and as models for endangered species. Much attention has also been paid to the control of reproduction, principally as a result of the considerable problem worldwide of pet overpopulation (Stavisky et al. 2012). However, there is no doubt that reproductive failure is common, and this may be attributed in part to the high degree of inbreeding (Leroy et al. 2015).

In normal circumstances the fertilisation/implantation rate is high – between 80% and 90% (Reynaud et al. 2006). The extent of infertility is, however, unknown. In the bitch, reduced fertility may not be fully appreciated because mating is relatively infrequent compared with other domestic species and because the majority of breeding animals are housed singly or in small groups. The situation is further complicated because 'fertility' often represents an owner's personal expectation of the reproductive performance of their own bitch or queen; this expectation may differ between a commercial breeding establishment and the owner of an individual pet. In addition, there are wide breed variations, particularly in litter size, which make it difficult to compare animals of different breeds.

It is with increasing frequency that both individual breeders and the managers of breeding colonies approach the veterinarian for help with breeding problems. Ultimately, this approach is because of the value of an individual animal that fails to reproduce or because of a concern for the decline in the productivity of a breeding colony. In the former case, continuation of a breeding line is the ultimate aim, whereas for the latter, greater productivity allows smaller numbers of breeding animals to be maintained. The breeding of large numbers of dogs and cats is common for pharmaceutical and biological laboratories and also for the production of working and assistance animals such as guide dogs for visually impaired people. Colony management usually requires not only a high output but also consistent production throughout the year.

The normal expectation of fertility in the bitch is a whelping rate in the region of 70% to 80% (England 1992), whereas the queen may rear between one and three litters per year (Stabenfeldt & Shille 1977). There are considerable variations from this, depending on age and breed. For the bitch the peak in reproductive efficiency occurs at approximately 3 years of age, with a decrease in the pregnancy rate from age 8 (Fig. 33.1), and a significant decline in the number of pups born in bitches aged 7 years and older (Blythe & England 1993). Blythe and England (1993) also demonstrated a variation in prolificacy between different breeds of dog. Similarly, in the cat, an age-related reduction in the number of litters per year and average litter size has been noted, which is most marked after 6 years of age (Lawler & Bebiak 1986). A study of 2400 pregnancies demonstrated that in older bitches, although fewer pups were born within each litter, there was also a lower incidence of stillbirth, such that overall the rate of decline in weaned pups was slower than the decline in the number of pups born (Phillips & England, unpublished observations). It should be remembered, however, that there are marked breed variations for normal litter size in both the dog and cat (Robinson 1973); these should be considered when an animal is presented because of alleged subfertility.

The investigation of infertility in the bitch is complicated by the fact that failure to conceive does not result in an immediate return to oestrus as occurs in polyoestrous species. However, the early diagnosis of pregnancy is now possible, using real-time ultrasonography and the measurement of plasma concentrations of relaxin. These methods allow improved investigations into why an individual animal fails to produce live offspring. Furthermore, studies have demonstrated the value of real-time ultrasound in documenting early pregnancy failure (England & Russo 2006).

As with other species, infertility in the bitch and queen may be categorised according to whether the cause is: (1) structural (including congenital, acquired, and neoplastic diseases); (2) functional (including endocrinological abnormalities); (3) infectious; or (4) managemental. The influence of the male should always be investigated; collection and evaluation of a semen sample provide a basic assessment of the male's fertility. In addition, attention should also be given to the mating routine because owners who are unfamiliar with normal mating behaviour may inadvertently be hindering conception. Examples of this are: the belief that ovulation shows a set number of days after the onset of prooestrus in the bitch and that only a single mating is necessary to induce ovulation in the queen.

The Bitch

Structural Abnormalities of the Bitch's Reproductive Tract

Congenital Abnormalities

Ovarian agenesis is rare, and does not cause infertility unless both ovaries are affected. In some cases, there may also be agenesis of the ipsilateral uterine tube and/or uterine horn, although the latter may occur with a normal ovary. Ovarian dysplasia has also been

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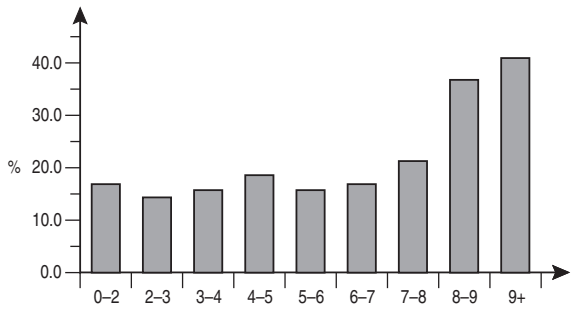


• **Fig. 33.1** The percentage of bitches not pregnant after mating to a fertile stud dog according to maternal age.

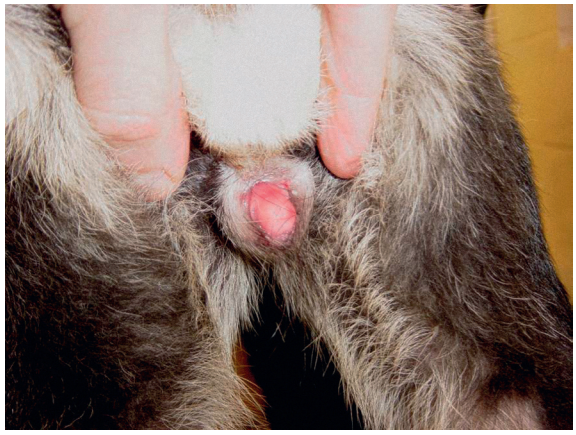
reported in a bitch with an abnormal number of chromosomes (Johnston et al. 1985), and evaluation of the karyotype may be useful when investigating the cause in these cases.

Bitches with uterine tube and/or uterine horn agenesis and normal ovaries usually exhibit typical oestrous behaviour but either fail to become pregnant (if there are bilateral lesions) or have small numbers of offspring (if there is a unilateral lesion). Diagnosis usually relies upon direct inspection of the reproductive tract via laparotomy or laparoscopy; the use of radiography after the injection of radiopaque contrast media into the uterus (Lagerstedt 1993) is not as useful in the bitch as for other species, particularly for demonstrating lesions of the uterine tube, which in this species is very small and rarely fills with contrast medium.

Other congenital anomalies of the tubular genital tract include segmental aplasia of the Müllerian duct system. These anomalies may be: (1) a failure of the paramesonephric ducts to form, which results in segmental uterine horn aplasia or absence of a uterine horn; (2) a failure of fusion of the caudal paramesonephric ducts so that a single lumen does not form, resulting in duplication of the uterus (didelphus) or cranial vaginal septation; or (3) a failure of the caudal paramesonephric ducts to fuse properly with the urogenital sinus, resulting in imperforate hymen. The aetiology of the condition is uncertain; however, administration of exogenous hormones during pregnancy may result in this type of abnormality. Complete aplasia of the vagina results in infertility and allows the accumulation of uterine fluid.

Anatomical abnormalities of the caudal reproductive tract are perhaps most common in bitches. Such congenital abnormalities may be considered to have five aetiologies: (1) they are the result of hypoplasia of the genital canal, which causes vestibulovaginal hypoplasia and a vaginal stricture of some length; (2) they are the result of poor or inadequate fusion of the Müllerian ducts to the urogenital sinus, causing annular fibrous strictures at this site; (3) they relate to tissue vestiges remaining at the vestibulovaginal junction, resulting in hymenal remnants or a complete hymen; (4) the incomplete fusion of the two Müllerian ducts may result in a double vagina or a vertical fibrous division within the vagina; or (5) they have imperfect joining of the genital folds and genital swellings resulting in vestibulovulvar hypoplasia.

Congenital abnormalities of the external genitalia are rare. Vulvar hypoplasia associated with perivulvar dermatitis has been described; the relationship between this condition and early neutering has not been fully established. The condition may be worsened if the bitch becomes obese because the perivulvar skin folds may then develop and cover the vulva. Masculinised female pups that have an abnormally shaped vulva may be produced after androgen or progestogen administration to the dam during pregnancy (see 'Habitual Abortion' later in this chapter).



• **Fig. 33.2** A 4-month-old intersex German Shepherd with clitoral hyperplasia and the presence of an os clitoris.

Intersex

Intersex animals have ambiguous genitalia. In the bitch, this is usually recognised because of an abnormal phenotypic sex appearance; externally, the animal appears female, but when it reaches puberty, the clitoris enlarges and male-like behaviour may develop (Fig. 33.2). Intersex animals may be classified as those with abnormalities of: (1) chromosomes, (2) gonads, or (3) phenotype; these conditions have been reviewed by Meyers-Wallen (1993).

Fetal mammals develop female genitalia unless there is the presence of a Y chromosome. The SRY gene (the sex-determining region on the Y chromosome) encodes the testis-determining factor. It is thought that SRY activates genes in the testis pathway and/or suppresses genes in the ovarian pathway (Meyers-Wallen 2006). The normal diploid number in the bitch is 78XX.

Abnormalities of chromosomal number include phenotypic females (XO or XXX), which have underdeveloped genitalia, and chimeras and mosaics that arise from two cell populations with different chromosome constituents. In chimeras and mosaics, there may be both ovarian and testicular tissue (true hermaphrodite); the phenotype of the animal depends on the amount of functional testicular tissue.

Animals with abnormalities of gonadal sex are those in which chromosomal and gonadal sex are dissimilar; such individuals are called 'sex-reversed'. XX sex reversal is inherited as an autosomal recessive trait in the American Cocker Spaniel and appears to be familial in other breeds. Affected animals may conform to one of three categories: (1) true hermaphrodites with one ovotestis, bilateral uterine tubes, and normal external female genitalia; (2) true hermaphrodites with ovotestes and/or epididymides and masculinised external male genitalia; and (3) XX males.

Animals with abnormalities of phenotypic sex are those in which chromosomal and gonadal sex is the same; the internal or external genitalia are ambiguous. Animals may be either female or male pseudohermaphrodites. Female pseudohermaphrodites generally occur as the result of androgen or progestogen administration during pregnancy; they have masculinisation of the external or internal genitalia but with two ovaries. The clinical appearance may vary from simple clitoral enlargement to almost male-like external genitalia. Progestogens administered during pregnancy have been most frequently implicated because these agents are used by some veterinarians to prevent alleged luteal deficiency (a presumed low progesterone output from the corpora lutea (CL) during pregnancy that results in pregnancy failure; often

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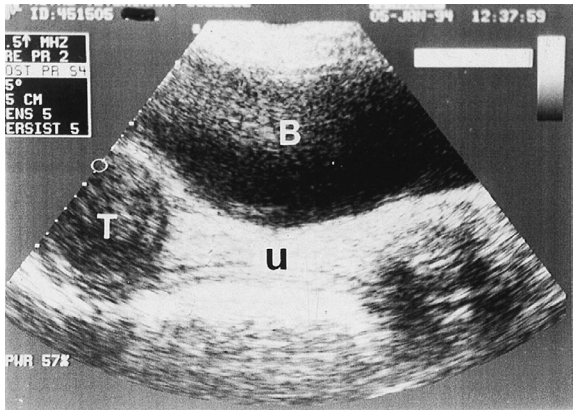
• **Fig. 33.3** Bitch with hyperplasia of the vagina during oestrus. The entire circumference of the vaginal mucosa is hyperplastic and protrudes from the vulva. Top is the dorsal commissure.



• **Fig. 33.4** Ultrasound image of the uterus of a bitch demonstrating a hypoechoic uterine tumour *(T)*. The uterus *(U)* lies dorsal to the bladder *(B)*.

also called habitual abortion). Male pseudohermaphrodites have testes, but the internal or external genitalia are feminised. This may be the result of failure of Müllerian duct regression or the failure of androgen-dependent masculinisation. In many cases the exact aetiology remains unknown. However, removal of the reproductive tract, including gonads, is usually necessary. After a gonadectomy, an enlarged clitoris may reduce in size, although clitoridectomy may be necessary if it continues to protrude from the vulva and be traumatised by the bitch.

Acquired Abnormalities

Acquired atrophy of the genitalia has been seen with neoplasia of the hypothalamus or pituitary; this is termed Fröhlich's syndrome but is very rare.

Other acquired abnormalities of the reproductive tract include endometrial hyperplasia (often cystic endometrial hyperplasia), pyometra (which are discussed later), and vaginal hyperplasia (Fig. 33.3). The latter condition may cause infertility by preventing mating. The aetiology is not clear; however, in some bitches the vaginal mucosa cranial to the urethral orifice becomes hyperplastic during an otherwise normal prooestrus and oestrus and may protrude from the vulva. In some cases, only the ventral mucosa protrudes as a simple tongue-shaped piece of tissue, whereas in other cases, it may involve the entire circumference of the vagina, resulting in the protrusion of a cylindrical-shaped mass through the vulvar lips. The hyperplasia appears to be an accentuated response to normal circulating oestrogen concentrations, which regresses at the beginning of the luteal phase (metoestrus/dioestrus). The hyperplasia commonly returns at the subsequent oestrus and, in many cases, appears to worsen at each subsequent cycle. Conservative therapy using emollient creams and topical antimicrobial agents is often sufficient, and recurrence may be prevented by performing an ovariectomy or ovariohysterectomy during the subsequent anoestrus. If the bitch is required for breeding, a submucosal resection may be performed during early oestrus. However, breeding from these bitches should be questioned because a familial tendency has been reported (Jones & Joshua 1982).

True vaginal prolapse is very rare in bitches but has been reported during oestrus (Schutte 1967), and chronic prolapse requiring hysteropexy has been observed during pregnancy (Memon et al. 1993).

Neoplasia

Ovarian tumours are uncommon in the bitch, accounting for approximately 1% of all neoplasms. There is an increased incidence of ovarian neoplasia in older dogs; the mean age of occurrence is 8 years (Withrow & Susaneck 1986). Ovarian tumours may be germ cell, epithelial, or sex cord stromal in origin. The most important are granulosa cell tumours, which may become very large and produce clinical signs related to the mass effect or ascites. They do not frequently metastasise, and are usually endocrinologically inactive; however, they may secrete: (1) progesterone, resulting in the absence of cyclicity, cystic endometrial hyperplasia, and pyometra; or (2) oestrogen, resulting in signs of persistent oestrus or possibly bone marrow suppression. Rarely, alopecia is a presenting clinical sign.

A less common tumour is the papillary cystadenocarcinoma, which may occur bilaterally. They commonly metastasise to the peritoneal lymphatics, producing obstruction and ascites, and have been found to be a consequence of prolonged administration of exogenous oestrogen.

The diagnosis of ovarian tumours is usually made on the basis of clinical signs, abdominal palpation, radiography, and ultrasonography. Ovariectomy or ovariohysterectomy may be curative if performed early. It is possible to induce seeding of a tumour at the time of surgery, but this is not common because of the presence of a well-defined ovarian bursa.

Uterine tumours are uncommon. The most frequently reported are fibroleiomyoma. These are discrete and nonmalignant, but haemorrhage may occur, resulting in a sanguineous vulvar discharge. These tumours seem only to develop in entire bitches and are very rarely diagnosed in ovariectomised animals. Uterine tumours may be diagnosed using real-time B-mode ultrasound (Fig. 33.4).

Tumours of the cervix are rare, but benign tumours of the vagina and vestibule are more common and include fibromata, fibroleiomyoma, and lipomata (Fig. 33.5). These often originate from the ventral vaginal floor cranial to the urethral orifice and may cause a local vaginitis and haemorrhage. Usually, they can be removed via an episiotomy; concurrent ovariectomy or ovariohysterectomy reduces the risk of recurrence.

The transmissible venereal tumour (TVT) (Fig. 33.6) affects the vagina and external genitalia of the bitch and the penis of the dog. Transmission of the tumour occurs at coitus when infected cells 'seed' the genital mucosa of the recipient. Autotransmission

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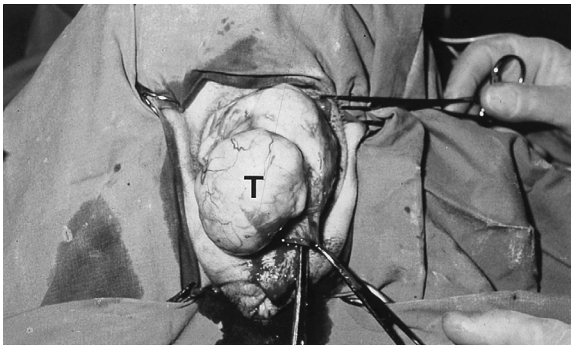

• **Fig. 33.5** Surgical removal of a large vaginal tumour *(T)* via an episiotomy.

• **Fig. 33.6** Transmissible venereal tumour within the vestibule of a bitch.

to the nasal and oral mucosa may occur by licking of the tumour. The lesions, which are often friable and multilobulated and may be single or multiple, generally reach their maximum size after 5 to 7 weeks; many then regress spontaneously within 6 months. The use of surgical debulking and various chemotherapeutic regimes has been described, including cyclophosphamide and vincristine, and radiation therapy. Intratumoural injection with vincristine and interleukin-2 appears to have a dramatic therapeutic effect (Den Otter et al. 2015). Such tumours are more common in tropical countries and in the UK and Europe generally only occur in imported animals.

Function Abnormalities of the Bitch's Reproductive Tract

Delayed Puberty and Prolonged Anoestrus

The age of puberty in the bitch ranges between 5 and 24 months, although this is influenced by breed, body weight, and environmental conditions. Bitches that do not reach puberty by 2.5 years of age are therefore considered to have delayed puberty. Because it is not uncommon for there to be few signs associated with the first oestrus, bitches that are thought to have delayed puberty may simply have had an unobserved oestrus. Failure to identify oestrus should also be considered in bitches that have prolonged interoestrous intervals. A high peripheral plasma progesterone concentration (> 2.0 ng/mL/ 6.5 nmol/l) demonstrates that ovulation has occurred within the last 60 days (i.e., a recent oestrus has been missed).

The normal interoestrous interval is between 26 and 36 weeks, and this is variable both within and between breeds and cannot be used to predict the next oestrus in an individual bitch. Therefore it is difficult to define prolonged anoestrus except that it is an interoestrous interval greater than that which was anticipated for a particular individual (normally > 8 months). The Basenji dog frequently exhibits its pubertal oestrus at approximately 300 days of age and thereafter cycles annually (Concannon 1993).

To investigate both delayed puberty and prolonged anoestrus, it is necessary to ensure that an oestrus has not been missed and that the animal's body weight and nutritional status are normal. Debilitating disease may result in a failure to cycle, as may the use of certain drugs, including progestogens, androgens, and anabolic steroids.

Some bitches with delayed puberty have an underlying chromosomal abnormality. Of these, many are phenotypically abnormal with a small, cranially positioned vulva and developing clitoral enlargement at puberty. A smaller proportion is phenotypically normal, but the abnormal complement of sex chromosomes results in ovarian hypoplasia or ovarian dysgenesis. Establishing a karyotype usually demonstrates chromosomal abnormalities such as 77XO, 79XXX, 79XXY, and 78XX/78XY.

Progesterone-producing ovarian cysts that prevent a return to oestrus have been described in the bitch, although these are very rare. In these cases the animal fails to return to oestrus and has persistently elevated plasma progesterone concentrations. Ovarian cystic structures may be found within the ovaries at ultrasound examination. Some bitches present with pyometra rather than an absence of cyclicity.

There has been considerable interest in the role of hypothyroidism as a cause of acyclicity in the bitch. The mechanism for this is not fully understood, although the administration of thyrotrophin-releasing hormone (TRH) causes the release of prolactin; therefore factors that affect TRH are likely to affect both thyroid function and prolactin secretion (Concannon 1986). It is rare for only reproductive signs to be present in dogs with hypothyroidism (there is more likely to be coat changes, weight gain, and lethargy); interestingly, the condition was shown not to be related to poor reproductive performance (Segalini et al. 2009), and so this must remain a dubious diagnosis in infertile dogs.

Induction of Oestrus

It is possible to induce cyclicity in bitches provided that there is no underlying disease. Unlike other domestic species, it is not possible to induce oestrus by shortening the luteal phase using prostaglandins because the luteal phase is followed by a variable, but prolonged, period of anoestrus. The most successful regime is to use the prolactin inhibitor (cabergoline); the precise mechanism is uncertain. A return to oestrus can be induced by daily administration of cabergoline starting in either the luteal phase or anoestrus, but fertility is best when treatment commences in anoestrus (Kutzler 2005). Exogenous gonadotrophin-releasing hormone (GnRH) may also be used to induce oestrus, using short acting implants or implants that are placed subcutaneously and then removed after a few days (Kutzler 2005).

For bitches with delayed puberty, it may be necessary to attempt oestrus induction using gonadotrophins should administration of prolactin inhibitors fail. Many protocols have used exogenous gonadotrophins for the induction of oestrus; commonly, low doses of equine chorionic gonadotrophin (20 IU/kg for 5 days) with a single administration of human chorionic gonadotrophin (500 IU on the fifth day) are recommended. Exogenous gonadotrophins should be used with care because Arnold et al (1989) showed that high doses and long treatment times could induce hyperoestrogenism,

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which prevents implantation and induces bone marrow suppression and death.

Silent Oestrus

Normal cyclical endocrine changes may occur in some bitches without obvious external signs of prooestrus or oestrus. It is possible that oestrous events are not observed by the owner because there is only slight vulvar swelling and minimal serosanguineous discharge or because the bitch is particularly fastidious. On some occasions, overt pseudopregnancy occurs in the absence of a preceding observed oestrus. However, ovulation can be confirmed by the measurement of peripheral plasma progesterone concentrations. If suspected, oestrus can be detected by the weekly examination of exfoliative vaginal cytology.

Split Oestrus

Occasionally, and most commonly at their first oestrus, bitches develop vulvar swelling and a serosanguineous vulvar discharge of a short duration. However, ovulation does not occur; follicles presumably regress and the signs of prooestrus disappear. A normal oestrus follows several weeks later. If the split oestrus (false oestrus) syndrome is recognised, it is important to ensure that mating occurs at the correct time in relation to ovulation. In some cases, the initial or subsequent oestrus is prolonged, and here induction of ovulation may be attempted using human chorionic gonadotrophin (hCG), although it can be difficult to be certain when to administer this preparation. This is most successful when administered at the peak of vaginal epithelial cell cornification. Occasionally, a split oestrus is not recognised, and the return to oestrus is mistaken for pyometra because of the development of a vulvar discharge.

Unpredictable Ovulation Time

Most bitches ovulate between 10 and 14 days after the onset of prooestrus. However, ovulation in normal bitches may occur as early as day 5, or as late as day 30, after the first signs of prooestrus (Fig. 33.7). In addition to this, there are large variations in the pattern of cyclical activity, thus bitches are not necessarily consistent between cycles (England et al. 1989). Infertility may therefore result because of attempted matings at inappropriate times in relation to ovulation (see 'Management Factors Affecting Fertility' later in this chapter).

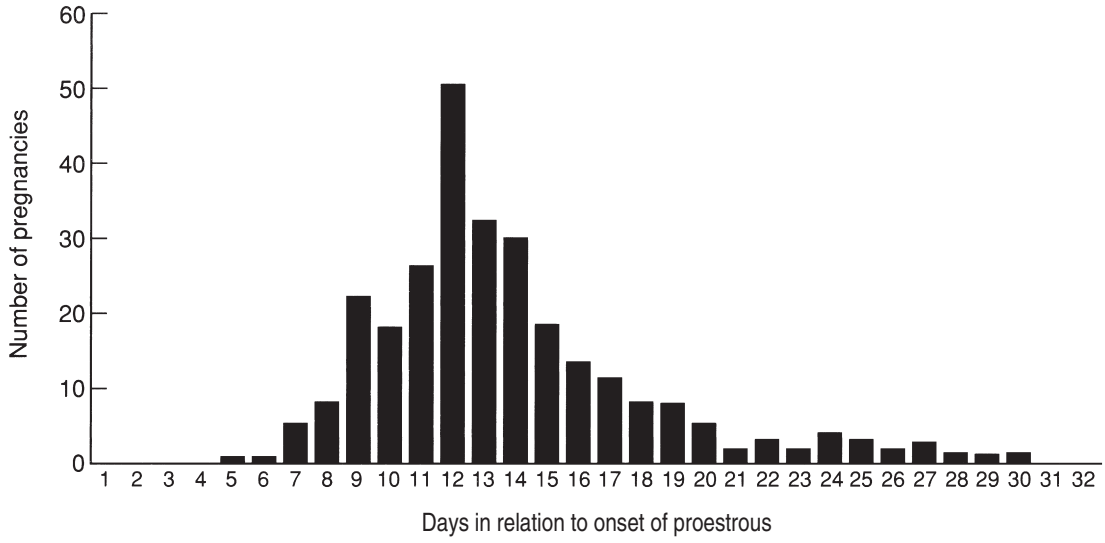
Prolonged Prooestrus/Oestrus

The normal interval between the onset of prooestrus and ovulation varies from 5 to 30 days. However, most bitches ovulate by Day 14 after the onset of prooestrus, and those that ovulate later than this are often considered to have prolonged oestrus. In fact, the majority of these animals are normal and do not require treatment but require careful assessment of the optimal mating time. Bitches in whom prooestrus or oestrus persists longer than 30 days (Wright 1990) may require treatment. The induction of ovulation may be attempted by the administration of hCG (20 IU/kg). It can be difficult to predict the correct time to administer hCG because its early use may result in either failure of response or possibly luteinisation without ovulation. Commonly, hCG is administered when more than 80% of exfoliated vaginal epithelial cells are anucleated.

Oestrogen-secreting follicular cysts are very rare in the bitch, but these may produce persistent oestrus. Similar clinical signs may be seen with oestrogen-secreting ovarian tumours, in which high concentrations of oestrogen may lead to bone marrow suppression, resulting in anaemia and thrombocytopenia. In such cases, treatment is by unilateral ovariectomy, although consideration should be given to the fact that the bitch may be normal or have a split oestrus syndrome.

Ovulation Failure

Until recently, the diagnosis of ovulation failure was most commonly made on the basis of a shortened interoestrous interval. However, after the introduction of routine monitoring of plasma progesterone concentrations, bitches that fail to ovulate have been detected. The incidence of ovulation failure has not been established, but it seems particularly common in German Shepherd



• **Fig. 33.7** The relationship between the calculated day of ovulation and the number of days from the onset of proestrous behaviour in 278 bitches (England, unpublished observations).

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and related breeds, sometimes manifesting as ovulation with a short luteal phase. Attempts at treatment may be made by the administration of hCG as described previously.

Ovarian Cysts

Cystic follicles (Fig. 33.8) and CL are very rare in the bitch; most ovarian cystic structures are of parabursal origin (Fig. 33.9), and although they may be detected by ultrasound and misinterpreted by the inexperienced clinician, they have no clinical significance because they are endocrinologically inactive.

• **Fig. 33.8** An ovary of a Labrador bitch demonstrating multiple follicular cysts.

• **Fig. 33.9** The reproductive tract of a bitch demonstrating multiple parabursal cysts adjacent to the ovary and proximal uterine horn.

True oestrogen-secreting follicular cysts produce persistent oestrus with vulvar discharge, and after several months, there may be flank alopecia and hyperkeratosis. Ultrasonographically, large fluid-filled follicles may be identified but must be carefully differentiated from normal follicles and early CL, both of which have central fluid-filled cavities. Some follicular cysts can be induced to luteinise by alternate day administration of hCG on three occasions. In others, such treatment does not succeed, but the cysts may respond to progestogen administration using hormones such as megestrol acetate. With the latter, there is an increased risk of pyometra because the progestogen is administered after a prolonged period of oestrogen priming. Ovariectomy may be necessary in cases that do not respond to exogenous hormone therapy.

Luteal cysts have been identified in postmortem studies; however, their significance is unknown. Burke (1986) suggested that they may secrete progesterone and produce prolonged anoestrus (sic) and cystic endometrial hyperplasia; in some cases, this may progress to pyometra.

Follicular and luteal cysts appear more commonly in older bitches. Some of these (often both types occur simultaneously) have been noted in the ovaries of aged bitches, which have a mucohaemorrhagic vulvar discharge, a condition referred to as metrorragie (Lesbouyries & Lagneau 1950). These bitches are often attractive to male dogs, but they will not allow coitus; a study of exfoliative vaginal cells demonstrates neutrophils, erythrocytes, and parabasal epithelial cells. This condition is often persistent and unresponsive to medical therapy such that ovariohysterectomy is usually the treatment of choice.

Premature Ovarian Failure

Premature ovarian failure has been suggested as a rare, but permanent, cause of anoestrus in previously normal bitches (Feldman & Nelson 1987). For an accurate diagnosis, investigation of the karyotype and measurement of plasma concentrations of gonadotrophins and thyroid hormone should be undertaken (Johnston 1989). In valuable breeding animals, oestrous-induction regimens may be contemplated; however, there is no information on the aetiology of the condition or on the efficacy of these treatments.

Habitual Abortion

There is little, other than anecdotal, evidence to suggest that habitual abortion is a clinical problem in the majority of breeds of dog, with the exception of the German Shepherd, in whom inadequate luteal function has been documented (Gunzel-Apel et al. 2006). Cases of abortion and resorption with normal progesterone concentrations are commonly documented using real-time B-mode ultrasound, although England (1992) suggested that there was no increased incidence in those bitches that had previously had reproductive disease. Most cases of abortion are probably related to an abnormal uterine environment (cystic endometrial hyperplasia), fetal defects, and/or the result of infectious agents.

The role of poor luteal function as a cause is less certain and appears unlikely. Although implicated by some workers, the minimum concentration of progesterone required to support the pregnancy is only 2 ng/mL (Concannon & Hansel 1977); i.e., it is very low. There is no doubt that the CL remain the principal source of progesterone production throughout gestation and that

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pregnancy may be terminated by ovariectomy or the induction of luteolysis at any stage. However, clinical problems appear only to occur in the German Shepherd (Gunzel-Apel et al. 2006) and related breeds or after oestrus-induction regimens and in one case of oophoritis (Nickel et al. 1991). In the author's experience, plasma progesterone concentrations of bitches with habitual abortion are not dissimilar to those of normal pregnant bitches (except for the German Shepherd dogs). Progesterone or progestogen supplementation during pregnancy may produce masculinised female pups and cryptorchid male pups and may possibly impair or delay parturition, resulting in fetal death. Progestogen therapy should be restricted to those cases in which a true luteal insufficiency has been diagnosed; this requires serial monitoring of progesterone concentrations.

Infectious Agents of the Bitch's Reproductive Tract

There are three categories of organism that may exert an influence upon fertility: (1) those that are known to have a specific pathogenical effect upon the reproductive tract; (2) those that are present in the normal environment and, under certain circumstances, can become opportunistic pathogens; and (3) those that cause systemic disease and exert their effect upon reproduction indirectly.

Normal Vaginal Bacterial Flora

There is a widespread belief among breeders and veterinarians that infertility, vaginitis, and fading puppy syndrome are caused by bacteria that inhabit the reproductive tract of the dog and bitch. This arose from the work of Stafseth et al (1937) and Hare and Fry (1938), who concluded that streptococci, especially beta-haemolytic types G and L, were responsible for infertility, abortion, anoestrus, and weak pups. With the advent of virus isolation techniques, several specific viruses have been identified, and it seems likely that the earlier work overemphasised the importance of the streptococci. These bacteria are now considered to be part of the normal commensal flora and probably invade subsequent to viral damage or are contaminants.

Many aerobic and anaerobic bacteria normally inhabit the vestibule and vagina of the healthy bitch, and the bacterial flora is normally mixed. The aerobic bacteria isolated from normal bitches include *Escherichia coli,* staphylococci, and streptococci; the anaerobic bacteria include *Prevotella* spp. and *Peptostreptococcus* spp. Mycoplasmas have been isolated from between 30% and 88% of normal bitches (Baba et al. 1983). Greater numbers of bacteria are found within the vestibule compared with the vagina; the uterus is normally sterile. The stage of the oestrous cycle may influence the bacterial flora because there is a significant increase in vaginal bacterial numbers when oestrogen concentrations are elevated (van Duijkeren 1992). Several authors have examined the vaginal bacterial flora of normal bitches and compared them with those of infertile bitches. These studies, which were reviewed by van Duijkeren (1992), showed that the bacterial species cultured from infertile bitches did not differ significantly from healthy bitches. Similarly, Hirsch and Wiger (1977) found that the organisms recovered from bitches with vaginal discharge were qualitatively the same as the normal bacterial flora, although the bacterial numbers were higher.

Therefore the results of microbiological examination of the reproductive tract of the bitch must be treated with caution because the simple isolation of bacteria from the vagina does not constitute a diagnosis of reproductive disease.

Opportunistic Pathogens

The bacterial species found in bitches with reproductive disease do not differ significantly from those found in healthy bitches. However, disease may result if the uterine or vaginal defence mechanisms are depressed, thereby allowing overgrowth of the normal commensals (Olson et al. 1986). Many of the normal vaginal inhabitants may become pathogens if a breakdown in local immunity occurs (van Duijkeren 1992). Some species of *E. coli* are more likely to be pathogenical because they possess particular adhesive capacity; these are called the uropathogenic *E. coli.*

Bacteria may enter the uterus during oestrus when the cervix is relaxed and could then cause infertility either directly by interfering with the zygote or by producing spermicidal factors. Bacteria may persist within the uterus after mating, particularly if the uterus is abnormal. It is clear that cystic endometrial hyperplasia can lead to mating-induced endometritis and reduced fertility, which can be improved by postmating antibiotic administration (England et al. 2012). Persistence of opportunistic pathogens, in the presence of endometrial hyperplasia, may lead to the later development of pyometra during the progesterone-dominant phase of the cycle.

If vaginal microbiological sampling reveals bacteria present in a pure growth or in very large numbers, then they may be considered significant, although pure growths of bacteria may also be isolated from normal dogs (Bjurstrom & Linde-Forsberg 1992). Those bacteria most commonly thought to be significant by some workers, but not this author, include *Pseudomonas* spp., *Proteus* spp., and some streptococci. Repeated culture after 1 week should be performed to confirm the diagnosis before attempting treatment. Appropriate antimicrobial therapy, based upon sensitivity tests, should only be administered after investigation of possible predisposing causes such as anatomical, neoplastic, or mechanical abnormalities of the vagina. In the author's experience, the presence of these organisms in pure growth is nearly always secondary to some other cause. Parenteral and topical administration has been advocated. It is noteworthy, though, that prolonged antibiotic treatment can lead to selection of pure growth of some bacteria, and so inappropriate antibiotic use may be the primary cause of this problem.

Mycoplasmas and ureaplasmas have been implicated in causing reproductive disease in the bitch (Lein 1986), although they are also frequently isolated in clinically normal animals with no evidence of reproductive tract disease. Mycoplasmal colonisation of the vagina has been demonstrated after prolonged treatment of bitches with oral ampicillin and potentiated sulphonamides (Strom & Linde-Forsberg 1993); this suggests that the widespread use of antimicrobial agents in healthy bitches should be avoided.

Specific Infections

Brucella Canis

Brucella canis is a Gram-positive bacterium that can produce abortion and infertility and result in significant economic losses for dog breeders. It is the only bacterium known to be a specific cause of infertility in the bitch. *Brucella* infertility was first reported in the United States but has subsequently been found in several countries worldwide. Barton (1977) found that between 1.5% and 6.6% of dogs in the United States had antibodies diagnostic

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of infection. Current rates may now be higher, and certainly they are in other countries; Keid et al (2017) found 42% of commercial breeding kennels in Brazil had at least one positive dog. *B. canis* is not present, except sporadically, in imported animals in the UK. *B. canis* can be transmitted in several ways, including contact with aborted fetal or placental tissue, contact with the vaginal discharge of infected bitches, venereal transmission, and congenital infection. The most common method of infection is venereal. Abortion occurs most commonly between days 45 and 55 of pregnancy; however, there may be early fetal resorption or the birth of stillborn or, more rarely, weak pups.

The isolation of the bacterium from blood or aborted tissue is diagnostic of the disease; however, there may be prolonged periods when the bitch is not bacteraemic, so that a negative blood culture does not rule out infection. Treatment can be difficult because of the intracellular nature of bacterial replication; prolonged infection and shedding is common (Reynes et al. 2012). Because a carrier state can occur and these animals may be potential sources of infection, they are best neutered to remove them from the breeding programme.

Toxoplasma Gondii

Toxoplasma gondii infection causes abortion, premature birth, stillbirth, and neonatal death. Surviving infected pups may carry the infection. The public health consequences of *Toxoplasma* infection should be considered whenever it is diagnosed. The condition is best diagnosed by examination of impression smears from dead neonates. Although clindamycin is often used to treat the condition in dogs and cats, most regimes appear to have little effect on the bradyzoite stage.

Canine Herpesvirus

Canine herpesvirus in adult male dogs generally produces mild clinical signs, limited to the respiratory or genital tract. However, the virus may cause resorption, abortion, and stillbirths. Viral recrudescence may occur at subsequent pregnancies such that the clinical problem may persist for some considerable time in a breeding programme.

Infection of the pregnant bitch results in placental lesions and the infection of fetuses. The infected placentae are macroscopically underdeveloped and possess small, greyish white foci characterised by focal degeneration, necrosis, and the presence of eosinophilic intranuclear inclusion bodies. Experimental infection during early pregnancy causes fetal death and mummification, whereas infection during midpregnancy results in abortion; infection during late pregnancy results in premature birth. The virus has also been recovered from vesicular lesions on the external genitalia of bitches. Variable-sized vesicles are commonly observed in the vestibule, and frequently these lesions are evident at the onset of prooestrus, suggesting that venereal transmission is probably important in adult dogs.

Pups may become infected at birth during passage through the vagina and subsequently die with characteristic widespread histological necrotising lesions. Pups that survive the illness may show persistent neurological disorders. Pups are only at risk while in utero and during the first 3 weeks of life; experimental attempts to produce the generalised disease in older pups have failed. In the pups, the disease is rapidly fatal and treatment is often unrewarding; symptomatic therapy is all that is available because specific antiviral agents are not efficacious. Recrudescent viral shedding from the vesicular lesions may be stimulated by the stress of pregnancy and parturition.

With respect to diagnosis, serology demonstrates exposure, not infection, and virus isolation of pathological examination of fetuses is required. Infection is becoming increasingly prevalent within Europe, and as a result, a vaccine has been licensed for use (Chabchoub et al. 2006). The vaccine contains canine herpesvirus glycoprotein subunits and is administered 1 to 2 weeks after mating and again 6 weeks later (i.e., 1–2 weeks before the expected date of whelping). Revaccination is recommended at each pregnancy. A high level of efficacy has been demonstrated, although protective levels of antibody appear to be present for only 3 months.

Canine Parvovirus

Canine parvovirus infection has been implicated by some breeders as a cause of infertility in their kennels. However, Meunier et al (1981) found that after the introduction of canine parvovirus to a kennel of 2000 brood bitches, the conception rate, incidence of stillbirths, average litter size, and average number of pups weaned per litter did not change. However, canine parvovirus may cause an acute generalised infection in pups and death after the time that maternally derived antibody wanes, often after weaning. More rarely, there is infection in neonates less than 2 weeks of age, which can occur as a consequence of uterine infection or as a result of exposure to the virus soon after birth. Certain premises may have significant problems with canine parvovirus as a result of subclinical infection maintaining a viral output that infects neonates shortly after birth. In these cases, it may be necessary to cease breeding to remove susceptible animals from the equation and to ensure thorough cleansing and vaccination of any other animals before introduction.

Canine Adenovirus

It is well established that infection with canine adenovirus during pregnancy may result in the birth of dead or weak pups that die within a few days of parturition. The disease is uncommon except in unvaccinated bitches. In most cases the virus is ingested by neonates and causes mortality from 3 weeks of age onwards. Virus shedding can persist for some considerable time, and the virus may persist in the environment for up to 10 days. Carrier bitches may act as a source of infection for pups. Bitches should be vaccinated before mating, and when this is undertaken, there is reasonable maternally derived antibody for up to 10 days after birth.

Canine Distemper Virus

Experimental exposure of pregnant bitches to canine distemper virus was found to produce either clinical illness in the bitch with subsequent abortion or subclinical infection of the bitch and the birth of clinically affected pups. This provides evidence for transplacental transmission, although the frequency of this under natural conditions is unknown but appears to be rare.

Cystic Endometrial Hyperplasia

Aetiology

Although the exact aetiology of cystic endometrial hyperplasia (CEH) and pyometra is uncertain, this syndrome is probably best categorised as an infectious cause of infertility, even though the role of the endocrine environment is significant. It has been suggested that cystic endometrial hyperplasia, which precedes pyometra as a clinical disease, may cause infertility by allowing the development of a mating-induced endometritis (Freeman et al. 2013), resulting in conception failure and embryonic resorption. The condition may be recognised by ultrasound examination of the uterus during

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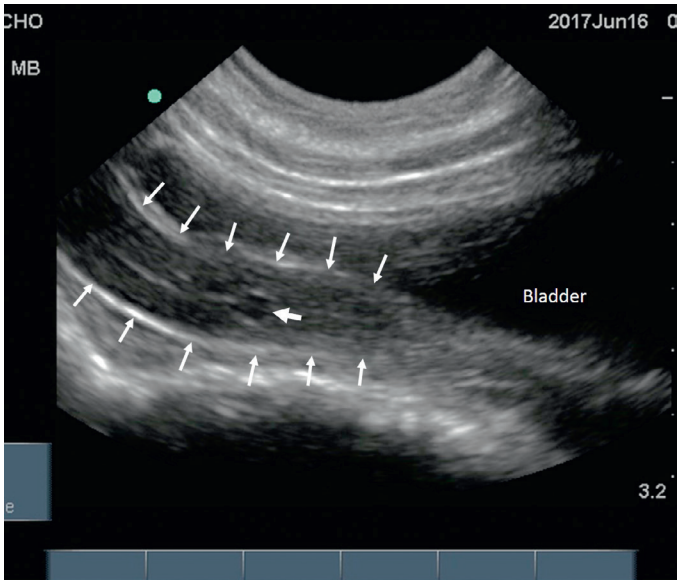

• **Fig. 33.10** Ultrasound image of the uterus of a bitch with cystic endometrial hyperplasia. The uterine body is seen in longitudinal plane *(arrows)* and a single central endometrial cyst is obvious *(large arrow)*.

the luteal phase. The detection of multiple small, focal, fluid-filled cystic regions within the endometrium is diagnostic (Fig. 33.10); endometrial biopsy is unnecessary. In many cases, cystic endometrial hyperplasia leads ultimately to pyometra, the incidence of which is high. In fact, pyometra is recognised as one of the common causes of illness and death in this species.

There has been considerable debate over the predisposing factors and the exact aetiology of CEH/pyometra. Most observers are of the opinion that CEH is a spontaneously occurring disease of middle aged or old bitches (Moxon et al. 2016). Continuing glandular hyperplasia occurs under the influence of progesterone and regresses at the end of the luteal phase. However, during the animal's life, there is progressive hyperplasia, which ultimately results in the development of pathological lesions termed cystic endometrial hyperplasia. The mean age of clinical pyometra is approximately 8 years, but it is not clear whether there is any association with parity or characteristics of the oestrous cycle. Pyometra is, however, very clearly a disease of the luteal phase, with most bitches showing clinical signs between 5 and 80 days after the end of oestrus; progesterone is the main hormone concerned with inducing uterine glandular hyperplasia.

Organisms isolated from the uterine fluid in cases of pyometra are those found as part of the normal vaginal and vulvar microflora. It is generally agreed that the predominant bacterium isolated is *Escherichia coli,* but has especial virulence factors. Sandholm et al (1975) found that the progesterone-sensitised endometrium and myometrium had an affinity for *E. coli.* These workers postulated that urinary tract infection was associated with pyometra, the uterus becoming infected during the early luteal phase when receptors for *E. coli* develop within the endometrium, thus enhancing the colonisation of the uterus with bacteria. It is likely that the long luteal phase of the bitch is an important contributory factor in the development of the condition because it has been shown that, in the cow, progesterone increases the susceptibility of the genital tract to infection. Brodey (1968) hypothesised that anogenital bacteria entered the uterus during oestrus and were able to proliferate during the luteal phase. This suggestion offers a plausible explanation of the aetiology because the condition is more likely to develop when there is cystic endometrial hyperplasia. A further factor that must be considered in the aetiology of pyometra is the use of exogenous reproductive hormones. Pyometra may be induced by the administration of exogenous progesterone or progestogens. The experimental use of oestrogens in the bitch does not usually result in the development of pyometra. However, oestrogens enhance the stimulatory effects of progesterone on the uterus. For this reason, when oestrogens are administered postmating to prevent conception, pyometra may be induced.

Clinical Signs

There is a wide range of clinical signs associated with pyometra in the bitch. When a complete history is available, it is usually found that the bitch was in oestrus a few weeks before the illness. In some cases, when there is a vulvar discharge, the owner may consider this to be a continuation of oestrus (Table 33.1).

If presented early, the general history is that the animal has been lethargic with a reduced appetite; increased thirst and vomiting are variable findings. Some bitches may be presented later when there is a vulvar discharge, which in some cases is associated with improvement in the general health. In other cases, the bitch remains unwell, and there is no discharge of pus. The bitch's abdomen may become distended, and she may be thought to be pregnant, although systemic illness is common. These cases generally end fatally, often within 14 to 21 days from the onset of clinical signs; the cervix remains closed throughout. Death may be due to toxaemia alone, or it may be associated with peritonitis due to rupture of the uterus. Occasionally, the cervix relaxes, and there is an outpouring of pus just before death.

In a further category, there may be intermittent opening of the cervix, with relative good health after the discharge of pus and malaise during the intervening periods.

Some cases of open cervix pyometra may persist for months, with a more or less continuous vulvar discharge. Body temperature may be normal or slightly elevated in cases of open cervix pyometra, whereas there is commonly an elevated body temperature in cases of closed cervix pyometra. In toxaemic patients the temperature may be subnormal.

The character of the vulvar discharge may vary considerably. Most often, it is of thin consistency and light chocolate brown in colour and has a characteristic odour. In other cases, it is yellow in colour, often blood-tinged, and varying from a watery to a creamy consistency. The vulva is generally enlarged, and there may be discoloration or scalding of the perivulvar tissues and perineum.

An increased thirst is commonly observed in advanced cases, which is due to reduced permeability for water in the distal convoluted tubule of the kidney. Renal dysfunction is probably caused by the formation of immune complexes. A small proportion of cases develop neurological signs, particularly ear twitching.

Diagnostic Features

Abdominal Palpation

Before examination, the animal should be given the opportunity to urinate and defecate. In cases of open cervix pyometra the uterine horns may be detected as thickened, often irregular, and slightly turgid structures from 1 to 3 cm in diameter. Their location within the abdomen is not generally altered from normal. Occasionally, some areas of the uterine horns are turgid and solid on palpation whereas others, which are distended by pus, may be indistinguishable from the surrounding bowel. Care must be taken not to confuse the colon with thickened uterine horns. In cases

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TABLE 33.1 Differential diagnosis of vulvar discharge in the bitch

Nature of the Discharge	Condition	History	Condition of the Vulva	Cytological Findings	Comments
Clear or straw-coloured	Oestrus	Expected in 'heat'	Swollen or slightly soft	LIEC, AEC, RBC, no WBC	Attractive to male
Mucoid	Metoestrus	Recent oestrus	Large but soft	PBC, SIEC, VSIEC, WBC	No malaise
Mucoid	Normal pregnancy	Pregnant/recent oestrus	Large but soft	PBC, SIEC, WBC	No malaise, does not threaten pregnancy
Purulent	Juvenile vaginitis	Before first 'heat'	Normal	PBC, SIEC, WBC	May respond to antibiotics but recurs Recovery after puberty
Purulent	Vaginitis	Variable but often excessive licking, attractive to male	Depends on the stage of the cycle	Depends on the stage of the cycle	Specific causes include: certain bacterial or viral infections, chemical irritation (urine), mechanical irritation (foreign body), neoplasia and anatomical abnormalities
Purulent/ haemorrhagic	Pyometra	Oestrus 2–8 weeks previously	Slightly swollen	WBC, SIEC, LIEC, RBC, bacteria, cell debris	Diagnosis using ultrasonography Often malaise
Purulent/ haemorrhagic	Metritis	Recent parturition	Large	Multinucleated cells, LIEC, uterine cells	Severe malaise
Haemorrhagic	Proestrus	Expected in 'heat'	Swollen	SIEC, LIEC, RBC, WBC	Attractive to male
Haemorrhagic	Oestrus	Expected in 'heat'	Swollen or slightly soft	LIEC, AEC, RBC, no WBC	Attractive to male
Haemorrhagic	Follicular cysts	Persistent discharge	Swollen	LIEC, RBC, ± WBC	No malaise, attractive to male, may develop bone marrow suppression
Haemorrhagic	Vaginal ulceration	Recent trauma or mating	Depends on the stage of the cycle	RBC, depends on the stage of the cycle	Rare, may start up to 2 weeks after mating
Haemorrhagic	Placental separation	Pregnant	Normal or slightly swollen	RBC, mucus	Ultrasound, radiography, etc., will confirm pregnancy
Haemorrhagic	Subinvolution of placental sites	Persistent discharge after whelping	Normal or slightly swollen	RBC, large polynucleated vacuolated cells	No malaise, refractory to treatment
Haemorrhagic	Transmissible venereal tumour	Not all countries of the world	Depends on the stage of the cycle	RBC, tumour cells?	Identification of tumour on vulva or in vagina confirms diagnosis
Haemorrhagic Cystitis	Cystitis	Frequent urination	Depends on the stage of the cycle	RBC, mucus	Small volumes of urine, dysuria
Haemorrhagic	Urinary tract neoplasia	Dysuria	Depends on the stage of the cycle	RBC, tumour cells?	Endoscopy may show origin of haemorrhage, positive contrast cystourethrography may be diagnostic
Haemorrhagic/ brown-coloured	Abortion	Pregnant	Slightly enlarged	RBC, mucus	Ultrasound shows uterus with similar appearance to postpartum
Green/brown-coloured	Parturition	Pregnant	Slightly swollen	RBC, SIEC, uterine cells	Painting, nest making, milk production
Green/brown-coloured	Dystocia, placental separation	Nonproductive straining	Slightly swollen	RBC, SIEC, uterine cells	Ultrasound will confirm pregnancy and fetal viability

PBC, parabasal cells; *SIEC,* small intermediate epithelial cells; *LIEC,* large intermediate epithelial cells; *AEC,* anuclear epithelial cells; *RBC,* erythrocytes; *WBC,* polymorphonuclear leucocytes; *VSIEC,* vacuolated small intermediate epithelial cells ('metoestrus cells').
Adapted from Allen & Renton 1982.

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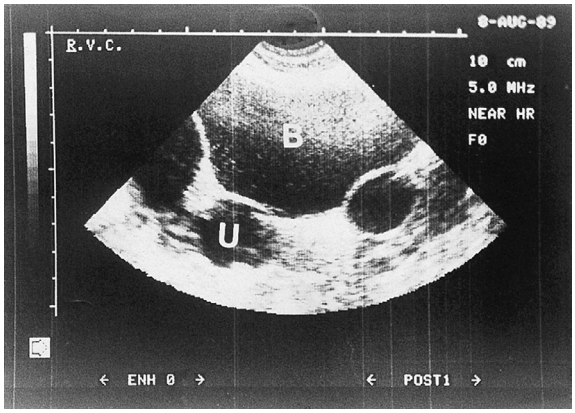
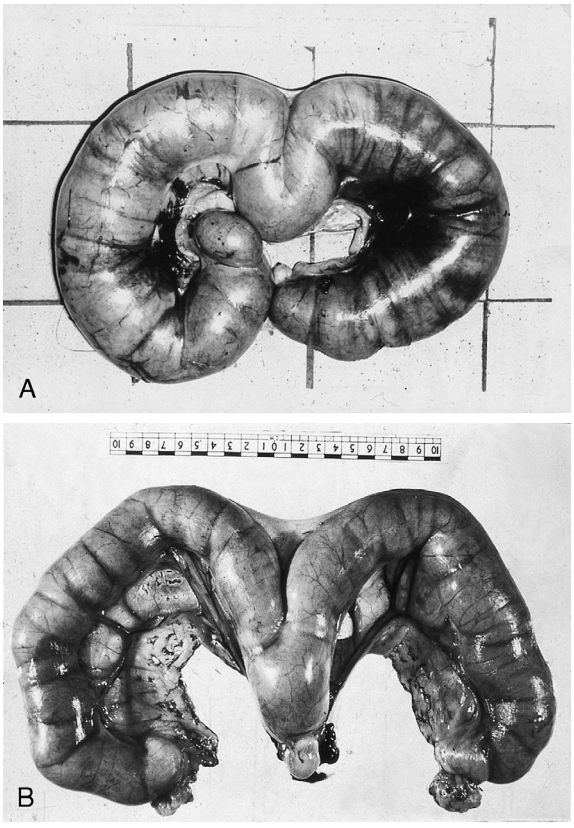


• **Fig. 33.11** Ultrasound image of the reproductive tract of a bitch with pyometra. The uterine horns *(U)* are distended with anechoic fluid and can be seen in three crosssectional planes dorsal to the bladder *(B)*.



• **Fig. 33.12** The reproductive tracts from two bitches with pyometra. Note the different degrees of distension.

of closed cervix pyometra, the degree of uterine distension may be greater, and there may be visible abdominal enlargement. In large or obese patients, abdominal palpation may not be possible.

Ultrasonography

Ultrasound is particularly valuable for detecting the uterus that is filled with fluid. The uterus has an increased diameter and may be folded upon itself so that several sections of each horn may be imaged in a single plane (Fig. 33.11). The diameter of the uterus may vary, depending on whether the cervix is open or closed. The uterine wall is usually relatively hypoechoic and is increased in thickness. The uterine lumen is usually grossly dilated with anechoic fluid, although small echogenic particles and mass lesions may be identified. The diagnosis is most simple when the diameter of the uterus increases to larger than that of the small intestine. Ultrasonography may be used to monitor cases of pyometra during treatment (England et al. 2007).

Radiography

The detection of a soft-tissue–opacity mass lesion within the caudal abdomen, causing cranial displacement of the small intestine and dorsal displacement of the colon, has been used for some time to indicate enlargement of the uterus. It should be remembered, however, that these findings are not specific for pyometra because early pregnancy has a similar radiographic appearance.

Haematology

The total number of leucocytes is frequently elevated in cases of pyometra, although the degree is much less marked in cases of open cervix pyometra compared with closed cervix pyometra. However, an elevated white cell count is not always present.

Rectal Examination

It may be possible to palpate the distended uterus per rectum using a finger, especially if slight backward pressure is applied to the abdominal wall.

Treatment

Ovariohysterectomy to remove the pus-distended uterus (Fig. 33.12) is the treatment of choice for pyometra. Bitches that are presented early in the course of the disease are usually a low surgical risk, and very high success rates have been reported. Lower success rates may occur when bitches are toxaemic. Intravenous fluid therapy is essential in all cases to ensure minimum renal toxic effects. Attention should also be paid to plasma electrolytes and acid–base status because complications associated with septicaemia, bacteraemia, and uraemia are common. Although the ideal situation is to administer intravenous, broad spectrum, antimicrobial agents and fluid therapy, it is not always possible to stabilise the patient before surgery.

If the condition is not life threatening and the animal is particularly valuable or when the clinical situation precludes surgery, medical treatment may be considered. Although repeated administration of prostaglandin will cause lysis of the CL and uterine contraction, it produces adverse effects (restlessness, pacing, hypersalivation, tachypnoea, vomiting, and diarrhoea) and is contraindicated if the cervix is closed. A better option is treatment with cabergoline (the prolactin inhibitor) with prostaglandin. Inhibition of prolactin removes endocrine support for the CL, so that progesterone concentrations decline (and closed cervix cases are converted to open cervix cases) before the administration of prostaglandin, which also cause luteolysis and uterine contractions. Treatment for 10 days, combined with fluid and antimicrobial therapy, is highly effective (England et al. 2007). Other medical regimes include the progesterone receptor antagonist (aglepristone), which blocks the action of progesterone. It is likely that, in the future, more complex treatment regimes, involving combinations of these three drugs (prolactin inhibitors, prostaglandins, progesterone receptor antagonists), will prove to be even more efficacious. Regardless of whether there is underlying endometrial hyperplasia, although the acute disease can be treated nonsurgically, there is a high rate of recurrence after the subsequent oestrus.

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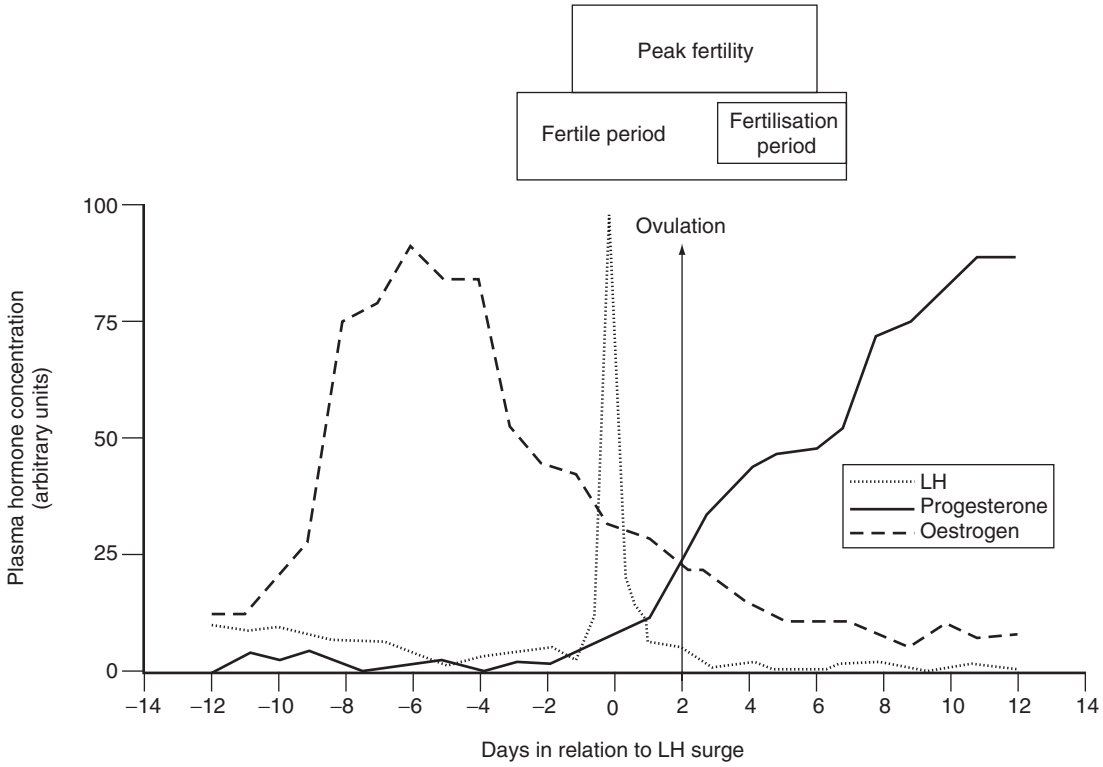

• **Fig. 33.13** Schematic representation of the changes in peripheral plasma hormones during proestrus, oestrus, and early metoestrus (dioestrus) and the relationship to the fertile period and the fertilisation period in the bitch.

Management Factors Affecting Fertility of the Bitch

The majority of bitches presented for fertility investigation are normal healthy fertile animals**,** whose apparent infertility is related to a misunderstanding of proper breeding management. In modern breeding protocols, the dog and bitch are often not allowed to display normal courtship behaviour because they are introduced when the owner considers that the time for mating is correct. This is usually based simply upon counting the number of days from the onset of vulvar swelling and the appearance of a serosanguineous vulvar discharge.

Although the majority of bitches ovulate between 10 and 14 days after the onset of prooestrus, this event may occur as early as Day 5 or as late as Day 30. In addition, bitches are not necessarily consistent between cycles (England et al. 1989). Therefore should a bitch be mated on Days 12 to 16 after the onset of prooestrus (which is common breeding practice), this may be inappropriate and result in a failure of conception.

The Fertile and Fertilisation Period

A surge in plasma LH concentration is the trigger for ovulation, which occurs 40 to 50 hours later. Ovulation is spontaneous, and eggs are ovulated as primary oocytes. The oocytes are immature at ovulation and must reach the metaphase of the second meiotic division after extrusion of the first polar body before fertilisation; this further stage of maturation takes 48 to 60 hours. Eggs remain fertilisable for a further 2 to 3 days; therefore, the time span over which fertilisation may occur, termed the 'fertilisation period', is between 4 and 7 days after the LH surge (i.e., between 2 and 5 days after ovulation) (Fig. 33.13).

Dog sperm can remain viable and fertile within the uterus and uterine tubes for at least 6 days or more (Doak et al. 1967). Therefore it is possible for matings that take place before the fertilisation period to result in conception. A second term may therefore be used, the 'fertile period', which differs from the 'fertilisation period' in that it encompasses the period of sperm survival within the female reproductive tract before ovulation and oocyte maturation. The fertile period extends from 3 days before until 7 days after the preovulatory LH surge and may be even longer for dogs with exceptional semen quality. Determination of the time to mate can therefore be assessed on the basis of the time of the LH surge or methods that may reliably indicate the 'fertilisation period' or the 'fertile period'.

Although it was initially thought that the LH surge occurred synchronously with the onset of standing oestrus, it was subsequently shown that this may occur between 3 days before and 9 days after the onset of oestrus. Therefore teasing the bitch has little value in determining the fertile period. Clinical assessment of the volume and colour of the vaginal discharge is similarly unreliable for determining the fertile period.

The Optimal Mating Time

The optimal time for mating is likely to be during or immediately before the fertilisation period, and the period of peak fertility for natural matings ranges from 1 day before to 5 to 6 days after the LH surge (see Fig. 33.13). Determination of the time to breed could therefore be based upon methods for estimating the time of the LH surge.

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TABLE 33.2 Plasma (serum) progesterone concentration in relation to stage of the bitch's cycle for the purposes of breeding management

Phase of the Cycle	Progesterone Concentration	Range
LH surge	≈ 2.5 ng/mL (8 nmol/l)	2.0–3.0 ng/mL
Ovulation	≈ 7 ng/mL (21 nmol/l)	5–9 ng/mL
Beginning of the fertilisation period	≈ 10 ng/mL (32 nmol/l)	8–15 ng/mL
Middle of the fertilisation period	≈ 15 ng/mL (45 nmol/l)	10–30 ng/mL
End of the fertilisation period	≈ 28 ng/mL (85 nmol/l)	25–40 ng/mL

Hormone Measurement

Measurement of plasma concentrations of LH is a reliable and accurate method of determining the optimum time to mate. However, there is no readily available commercial assay for canine LH. A further key challenge in measuring LH is the fact that concentrations peak and return to basal values within a short period of time, necessitating daily sampling.

Plasma progesterone concentrations begin to increase towards the end of prooestrus at the time of the LH surge. Progesterone is produced by luteinising follicles, and therefore, serial monitoring of plasma progesterone concentrations allows anticipation of ovulation. Because progesterone concentrations rise slowly before ovulation, daily sampling is not necessary. Commercial test kits designed to measure the concentration of plasma progesterone by ELISA are available. Plasma progesterone concentrations in relation to the different phases of the cycle are shown in Table 33.2.

Vaginal Cytology

Examination of exfoliative vaginal cells is commonly used to monitor the oestrous cycle. During prooestrus, increased plasma oestrogen concentrations cause thickening of the vaginal mucosa, which becomes a keratinised squamous epithelium. Vaginal epithelial cells may be collected either using a moistened swab or by aspiration. The relative proportions of different types of epithelial cells can be used as a marker of the endocrine environment. Several methods for staining of the cells and various indices of cornification and keratinisation have been suggested as markers for the stage of the cycle. In general, the fertile period can be predicted by calculating the percentage of epithelial cells that appear cornified using a modified Wright–Giemsa stain, although staining with a modified trichrome stain, allowing an assessment of the percentage of keratinised cells, has been suggested as being most useful. A schematic representation of the changes of exfoliative vaginal cells is given in Chapter 1.

Polymorphonuclear leucocytes are absent from the vaginal smear during oestrus because the keratinised epithelium is impervious to these cells. Their reappearance during late oestrus reflects the breakdown of this epithelium. The return of polymorphonuclear leucocytes to the vaginal smear has been used by some workers as an indicator of the time of optimum fertility; also, it is easy to document.

Many workers suggest that breeding should be attempted throughout the period when more than 80% of epithelial cells are cornified (Fig. 33.14). Although this is a good guide, some bitches reach peak values of only 60% cornification, whereas in others, there may be two peaks of cornification (van der Holst & Best 1976). Some bitches demonstrate poor cellular changes in the vaginal smear, and typical metoestrous cells may be found during prooestrus. Allen (1985) noted that polymorphonuclear leucocytes may be found throughout the entire period of oestrus; the extent of these variations has not been quantified. Planning of mating, based on evaluation of the anuclear cell index of cells aspirated from the cranial vagina, was found to increase the pregnancy rate and litter size of a group of bitches compared with a similar group mated only on the basis of the onset of prooestrus (England 1992). The technique is particularly useful for bitches with irregular oestrous cycles and those with a prolonged prooestrus or oestrus.

Vaginal Endoscopy

Vaginoscopy is the technique of examination of the vaginal mucosa using a rigid endoscope. Vaginoscopic assessment is based upon observation of the mucosal fold contours and profiles and the mucosal colour (Lindsay 1983). Enlarged oedematous pink or pinky/white mucosal folds are present during prooestrus and oestrus. Progressive shrinking of these folds is accompanied by pallor; such effects are probably the result of an abrupt withdrawal of the water-retaining effect of oestrogen during its preovulatory decline. Subsequently, mucosal shrinkage is accompanied by wrinkling of the mucosal folds, which become distinctly angulated and dense cream to white in colour. These gross changes have been used to assess the fertile period (Moxon et al. 2012).

Vaginoscopic evaluation is based upon assessment of the appearance of the vaginal wall (the mucosal fold contours and profiles, the colour of the mucosa, and the presence of any fluid) and the changes in this appearance at specific times of the oestrous cycle. A specific scoring system was devised by Lindsay and others (1988) and Jeffcoate and Lindsay (1989) and has been modified by this author as follows (Fig. 33.15):

- **Inactive phase (I)**: This phase is characterised by a thin, red, dry mucosa with low and flattened mucosal folds.
- **Oedematous phase (O)**: This phase is characterised by a thickened and oedematous mucosa, that appears turgidly swollen, rounded, and grey/white in colour.
- **Shrinkage phase (S)**: This phase is characterised by a thickened mucosa, which is normally white in colour but in which there is reduced turgidity with progressive furrowing, wrinkling, and indentations. The mucosal fold profile is, however, still rounded rather than angular. The progressive nature of this phase means that the early changes are designated 'S1' and the later changes 'S2'.
- **Angulated phase (A)**: This phase is characterised by a thickened mucosa, which is normally white in colour, but in which there is significant reduced turgidity to the extent that, in profile, the mucosal folds have progressive shrinkage and angulation, such that the peaks are sharp-tipped and irregular in appearance. The mucosa is wrinkled and shrunken in appearance. The progressive nature of this phase means that the early changes are designated 'A1' and the later changes 'A2' and 'A3'.
- **Declining phase (D)**: This phase is characterised by a progressive decline in the size of the mucosal fold profile. Early in the phase (designated 'D1'), there is a flaccid appearance to the mucosal fold profile. Subsequently, the folds become more rounded ('D2'), and there is sloughing of the cornified layers of

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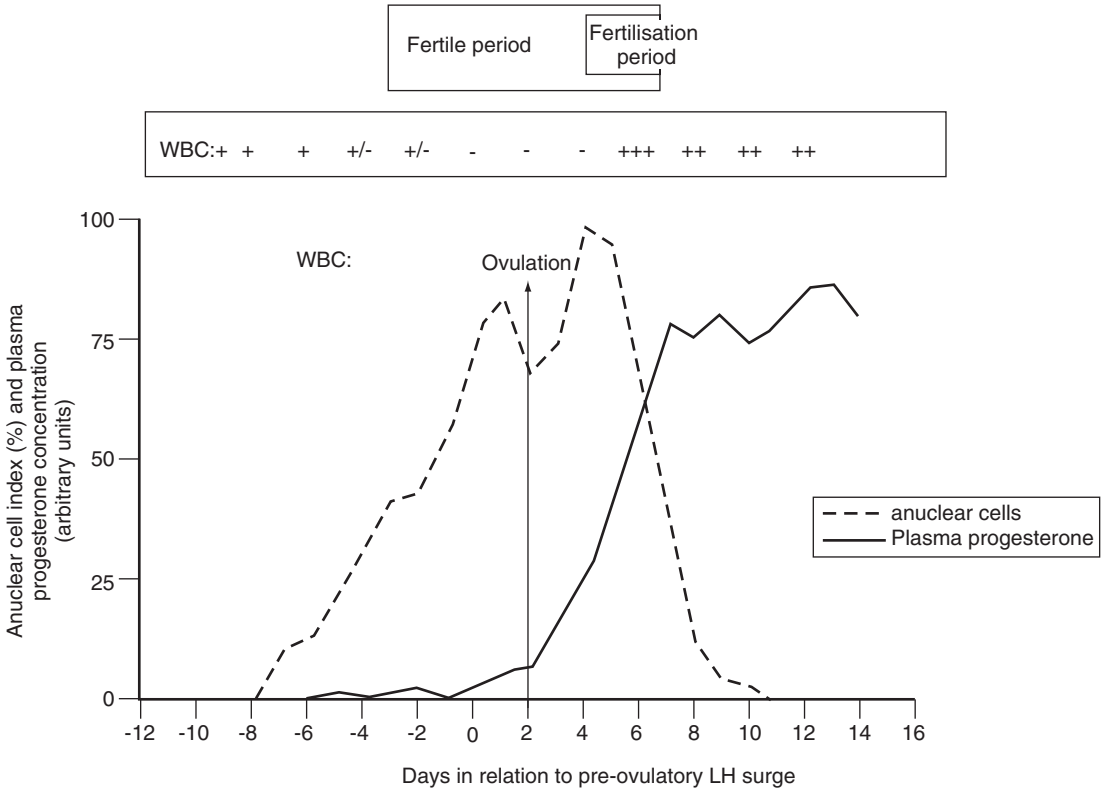
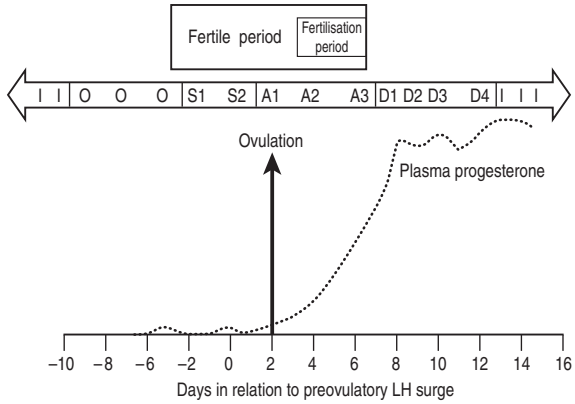


• **Fig. 33.14** Schematic representation of the changes in the percentage of anuclear epithelial cells in relation to ovulation and the fertile period in the bitch.



• **Fig. 33.15** Schematic representation of the relationship between the vaginoscopic score and the time of ovulation, the fertile and fertilisation periods, and plasma progesterone concentrations in bitches (see text for description of the various vaginoscopic scores).

the epithelium ('D2' and 'D3'), resulting in a thin mucosa of variegated colour with flattened folds and a rosette-appearance to the mucosa ('D4').

- **Inactive phase (I)**: The decline phase is followed by a return to a phase characterised by a thin, red and dry mucosa**,** with low and flattened mucosal folds. This appearance is also designated 'I'. There may be more debris present at this stage than found in phase I before the onset of prooestrus.

A clear relationship is present between the appearance of the vaginal wall and the underlying endocrinology (see Fig. 33.15). The specific timing of some events is variable**,** but a good correlation has been demonstrated between the onset of ovulation and phase A1 and a good relation between the fertilisation period and phases A1 through A3. Generally, the onset of the fertile period can be detected by observing the onset of mucosal shrinkage without excessive angulations, although gross shrinkage of entire mucosal folds with obvious angulation (phase A2 and A3) is characteristic of the fertilisation period (see Fig. 33.15). Breeding is best planned approximately 4 days after the first detected mucosal shrinkage or at the onset of the period of obvious angulation of mucosal folds. The end of the fertilisation period can be detected by observing sloughing of the vaginal epithelium (see previous mention) and development of a variegated appearance to the colour of the mucosal surface.

Ultrasound Examination

Real-time B-mode ultrasound imaging has been used in several species to monitor follicular growth and to identify the time of ovulation. Imaging of the bitch's ovaries and the detection of ovulation have now been reported in some detail (England et al. 2009). Detection of follicles with ultrasound can be difficult in obese animals when there is significant fat in the ovarian bursa. Even in thin females ovulation is quite difficult to detect because follicles do not collapse and because CL have central, fluid-filled cavities unoccupied by luteal tissue (Fig. 33.16). Regular (probably daily) examination is required to detect ovulation, and for this reason, ultrasound is not a technique that has found widespread clinical application.

Study of the Cervicovaginal Secretion

Variations in the electrical resistance of the vaginal secretion during the oestrous cycle have been described. In dogs, however,

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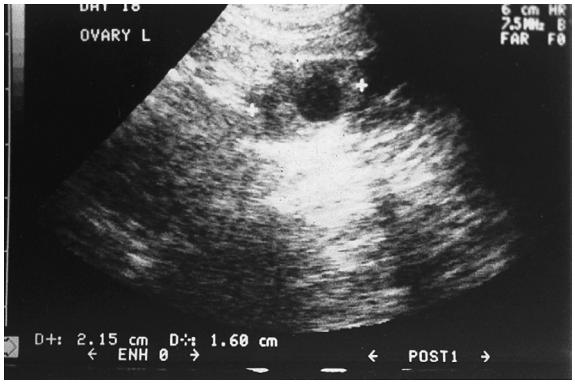

• **Fig. 33.16** Ultrasound image of an ovary of a bitch during metoestrus (dioestrus). A single large cavitated anechoic corpus luteum can be seen within the ovary *(crosses)*.

• **Fig. 33.17** Photomicrograph of the cervicovaginal fluid collected from a bitch during oestrus. Crystallisation of the mucus has resulted in the formation of a fern-like pattern.

the technique appears to have limited value and, in the author's experience, is not clinically useful. It is, however, used commercially for the detection of the insemination time of the fox (Fougner 1989).

Van der Holst and Best (1976) suggested that the amount of glucose within the vaginal discharge was a useful indicator of the optimum time of breeding. The principle of this technique is related to a change in the pH of vaginal secretion because glucose is liberated from carboglutelin, which is then converted into lactic acid (Vogel & van der Holst 1973). Although initial results were promising, this technique has not found clinical acceptance, presumably because of individual bitch variations.

Crystallisation of mucus collected from the anterior vagina has been described in the bitch (Fig. 33.17), occurring after the peak in plasma oestrogen concentrations (England & Allen 1989). Assessment of the mucus may be useful when combined with vaginal cytology for determining the optimal mating time.

Vulvar Softening

During prooestrus, the vulva becomes enlarged and turgid. There is often a distinct softening and decrease in swelling after the decline in plasma oestrogen, which occurs at approximately the day of the preovulatory LH surge. This method is imprecise but is probably the single clinical event that has proved to be useful for assessing the optimal mating time.

Cat

Structural Abnormalities of the Queen's Reproductive Tract

Congenital Abnormalities

The range of congenital abnormalities of the reproductive tract of the queen is similar to that of the bitch. Ovarian agenesis is rare and results in permanent anoestrus and infertility. Small ovarian remnants containing fibrous tissue may be identified at laparotomy or laparoscopy. Similar to the bitch, the establishment of a karyotype may be useful in the investigation of these cases.

Ovarian hypoplasia is also rare in the queen, although phenotypically normal queens may have nonfunctional ovaries secondary to chromosomal abnormalities.

There are a small number of reports of aplasia of the tubular genital tract and unilateral and/or bilateral agenesis of the uterine tube and uterine horn. It is not uncommon for these lesions to be associated with absence of the ipsilateral kidney and ureter. The queen may be fertile when the abnormality is unilateral, although litter size is often reduced. In many cases, these lesions are only diagnosed at exploratory laparotomy.

Vaginal aplasia may result in the retention of uterine fluid and cause endometrial changes and infertility. The uterus may be so large that it can be detected by palpation; otherwise, ultrasonography will demonstrate a tubular fluid-filled uterus present within the caudal abdomen. In rare cases, simple vaginal bands or strictures may be identified and can be manually broken down, allowing normal coitus.

Disease of the caudal reproductive tract is rare. Vulvar and vaginal atresia may occur separately or simultaneously. Small labia**,** with or without stenosis of the vestibule, are observed in the former, whereas stenosis of the vagina is observed in the latter.

Intersex

Female cats with ambiguous genitalia may be seen in clinical practice. In many cases, the external genitalia are underdeveloped until puberty, when the animal may demonstrate male-like behaviour. Clitoral enlargement has not been reported. An exact diagnosis may not always be reached; however, removal of the reproductive tract, including gonads, is necessary in most cases. Animals may be classified as having abnormalities of chromosomal, gonadal, or phenotypic sex; a full description of this is described earlier in the chapter for the bitch.

Acquired Abnormalities

Acquired abnormalities of the reproductive tract of the queen are rare. Occasionally, ovariobursal adhesions of unknown aetiology are identified, but these are usually unilateral and cause reduced fertility rather than infertility. The most common acquired abnormality is pyometra (see later in this chapter), although hydrometra has also been reported.

Neoplasia

Ovarian tumours are uncommon in the queen. They generally reach a large size before diagnosis and are not frequently metastatic, often being granulosa–theca cell tumours. These may be endocrinologically active and result in clinical signs of persistent oestrus, cystic endometrial hyperplasia, and bilaterally symmetrical alopecia, depending on whether they produce oestrogen or progesterone, respectively. The treatment of choice is ovariectomy. Malignant

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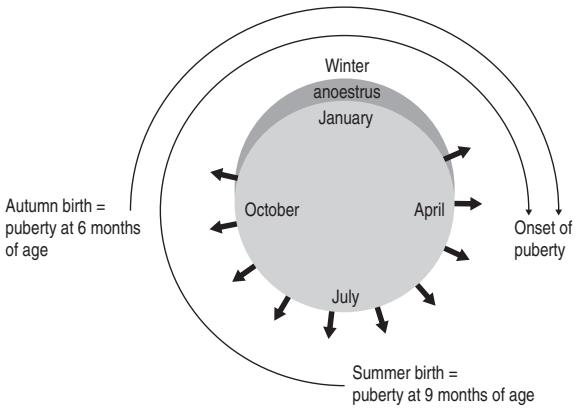


• **Fig. 33.18** Schematic representation of seasonal polyoestrous cyclicity in the queen *(large arrows represent oestrus)*, which show the variation of age at puberty depending on time of birth.

endometrial adenocarcinoma is the most common uterine neoplasm, although benign tumours have also been reported. These tumours may be associated with a persistent haemorrhagic vulvar discharge and straining. In all cases, ovariohysterectomy is curative, provided that metastases have not developed. Tumours of the vagina include pedunculated leiomyomata and fibromata. These are rare and generally produce clinical signs of vaginitis and straining to defecate because they may impinge upon the colon and rectum. Local excision is usually curative, and ovariohysterectomy may reduce the risk of recurrence.

Functional Abnormalities of the Queen's Reproductive Tract

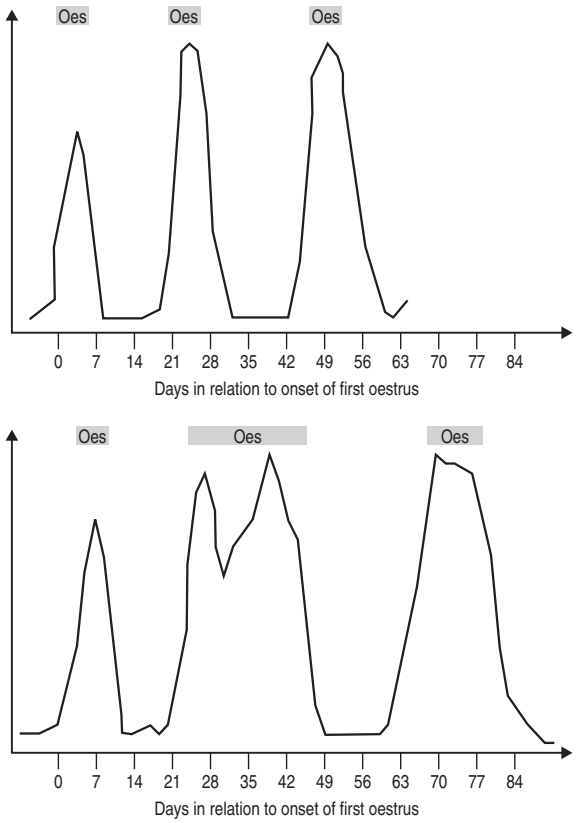
Delayed Puberty and Prolonged Anoestrus

The onset of puberty in the queen is influenced by both body weight and the season of birth because, normally, the queen is a seasonally polyoestrous breeder. In most cases, puberty occurs in the spring; as a consequence those animals born in autumn may exhibit their first oestrus at 6 months of age, and those born in the summer reach puberty at 9 months of age (Fig. 33.18). However, queens born in winter or spring may not exhibit puberty until 12 months of age (Goodrowe et al. 1989).

Queens that do not reach puberty by their second spring are considered to have delayed puberty. In the northern hemisphere, queens generally commence oestrous behaviour in January or February, an event that is dependent on photoperiod. Prolonged anoestrus may be associated with systemic disease, poor nutrition, or a high parasite burden. If these factors are eliminated, 14 hours of daylight should abolish anoestrus in the healthy normal queen. Attention should also be given to whether progestogens have been used for the control of dermatological or behavioural problems because their use will prevent oestrus.

Induction of Oestrus

When it is necessary to breed the anoestrous queen, it may be possible to induce cyclicity provided that there is no underlying disease by ensuring an appropriate day length (14 hours of light per day). When this fails, induction of oestrus can be attempted by the use of a depot GnRH preparation (deslorelin), followed by hCG (which is LH-like in activity) given when there is maximum cornification of vaginal epithelial cells; this is done for the purpose of ensuring ovulation (Zambelli et al. 2015).



• **Fig. 33.19** Plasma oestrogen in normal cycling queen *(top)* and queen with prolonged oestrus *(bottom)*; the period of standing oestrus (Oes) in these unmated queens is shown by the grey bar.

Silent Oestrus

Cases of silent oestrus are usually identified in queens that are low in the hierarchy of a cat colony. The incidence of silent oestrus is unknown, and the diagnosis is made because of the absence of behavioural oestrus when endocrinological events are normal. Cases are best evaluated by the study of vaginal cytology to demonstrate oestrus (see later), or removal of the queen to a new environment, or the induction of oestrus. Occasionally, the condition occurs in queens that are lactating, although more commonly cats are anoestrus at this time.

Prolonged Oestrus

Some queens appear to have a behavioural oestrus that persists for the duration of up to two follicular cycles (Fig. 33.19). In these cases, oestrogen concentrations remain high, presumably the result of overlapping follicular waves in the absence of ovulation. The condition is more common in oriental-type breeds. The fertility of these cycles is uncertain, although it is likely that fertility may be reduced because of inappropriate mating time and resultant failure of ovulation. In most cases, treatment is not warranted because prolonged oestrus is generally a sporadic occurrence.

Ovulatory Failure

Cats are induced ovulators, with oocytes being fertilisable at ovulation after mating or artificial stimulation of the genitalia. Copulation produces a rapid pituitary-mediated release of LH, and multiple

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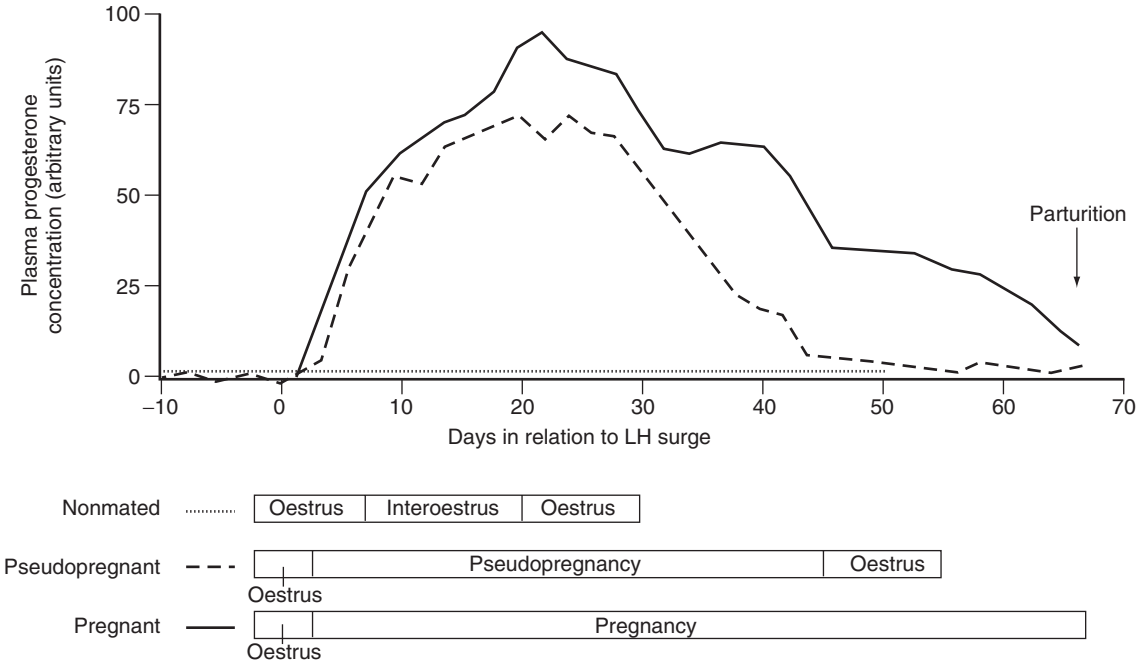

• **Fig. 33.20** Schematic representation of the changes in peripheral plasma progesterone concentrations in the nonmated, pseudopregnant, and pregnant queen. The hormone profiles are plotted in relation to the time of the LH surge for the mated animals and the onset of oestrus in the unmated animals.

copulations usually are required to ensure that ovulation occurs. Failure of ovulation may occur if the queen is not mated a sufficient number of times. However, approximately 90% of queens ovulate if mated three times at 4-hour intervals within the first 3 days of oestrus (Schmidt 1986). There is a reduced magnitude of LH release after matings that occur later into oestrus. Therefore breeding on the first few days of oestrous behaviour ensures the greatest success. Ovulatory failure may be diagnosed by a return to oestrus after 3 weeks (cats that ovulate but fail to become pregnant enter pseudopregnancy and have a delayed return to oestrus) and by measurement of plasma progesterone concentrations that remain basal after cessation of behavioural oestrus. Ovulation may be facilitated by ensuring that sufficient matings occur at the beginning of oestrus or by the administration of a single dose of hCG (500 IU) on the first day of oestrus; the hCG mimics the mating-induced LH surge.

Pseudopregnancy

Although, in the bitch, pseudopregnancy is not a cause of infertility, in the queen, pseudopregnancy does cause a delay to the return to oestrus. Nonfertile matings or occasional spontaneous ovulations (Lawler et al. 1993) result in the formation of CL, which secrete progesterone. During the life span of the CL, the queen does not demonstrate oestrous activity, resulting in interoestrous intervals of between 35 and 70 days, with an average of 45 days. Diagnosis of the condition is based upon the demonstration of elevated concentrations of plasma progesterone (Fig. 33.20). Late in the breeding season, pseudopregnancy may be followed by anoestrus rather than a return to oestrus.

Ovarian Cysts

In the queen, cystic structures can be associated with the ovaries. However, the majority of these are not of ovarian origin, being remnants of mesonephric and rete tubules. These cysts are endocrinologically inactive and do not produce clinical signs. True follicular cysts, associated with hyperoestrogenism, have been reported and may be associated with prolonged oestrus. Diagnosis may be made on the basis of clinical signs, on measurement of plasma oestrogen concentrations, or by the demonstration of persistent cornification of vaginal epithelial cells. hCG may be administered in an attempt to induce ovulation; however, in most cases, either ovariectomy or the use of progestogens to suppress the clinical signs is necessary.

Premature Ovarian Failure

Premature ovarian failure has been suggested as a cause of permanent anoestrus in previously fertile queens, which results in a shortening of their reproductive life. The condition is difficult to confirm, and diagnosis relies upon elimination of other causes of anoestrus. In valuable breeding animals, oestrus-induction regimes may be contemplated; however, there is no information on the efficacy of these treatments.

Habitual Abortion

There is little nonanecdotal evidence to show that habitual abortion occurs in the queen; however, the condition is occasionally diagnosed and treated. In many cases, it is suggested that habitual abortion is the result of progesterone deficiency, although evidence for this is lacking. As in the bitch, the minimum plasma concentration of progesterone required to maintain pregnancy is approximately 1 to 2 ng/mL, with ovarian-derived progesterone being the major source throughout pregnancy (Verstegen et al. 1993). The administration of progesterone or progestogens has been advocated to prevent habitual abortion; however, this therapy is empirical and suffers the risk of prolonging gestation and producing masculinised female kittens and cryptorchid male kittens.

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Progestogen administration should be limited to those cases in which a true luteal insufficiency has been diagnosed.

Infectious Agents Affecting the Queen's Reproductive Tract

There are no reported venereal-transmitted bacterial infections or specific genital infections that are recognised as causes of infertility in cats. However, there are several opportunistic pathogens and specific infectious agents that may have a direct effect upon fertility.

Opportunistic Pathogens

Many aerobic and anaerobic bacteria normally inhabit the vestibule and vagina of queens. It has been suggested that they may enter the uterus at mating and subsequently cause abortion because the progesterone-dominant uterine environment allows them to proliferate. It is not clear, however, whether bacteria isolated from aborted fetuses have caused the abortion or whether they have invaded the uterus after dilatation of the cervix at the time of the abortion. Bacteria commonly isolated include *E. coli,* staphylococci, streptococci, salmonellae, and mycobacteria. Immediately before an abortion, the queen may become pyrexic and lethargic. Treatment includes the administration of broad spectrum antimicrobial agents, fluid therapy, and drugs to stimulate uterine evacuation. Hysterotomy to remove fetal tissue is rarely necessary, although ovariohysterectomy may be required should a severe metritis develop.

Specific Infection

Feline Leukaemia Virus

Feline leukaemia virus (FeLV) has been implicated in a variety of clinical syndromes including infertility, embryonic resorption, and abortion. FeLV is believed to be the single most common cause of infertility in the queen (Jarrett 1985). Fetal resorption is seen frequently, although abortion and the birth of permanently infected kittens also occur. The aetiology of the reproductive disease is uncertain and, although it is known that the virus may cross the placenta, one possibility is that secondary bacterial infections occur because of FeLV-induced immunosuppression (Jarrett 1985). Diagnosis of FeLV infection may be achieved by virus isolation, immunofluorescence, or an ELISA method. Owners should be discouraged from breeding from FeLV-positive queens because all offspring are born persistently infected. These kittens usually develop a FeLV-related disease soon after birth. Vaccines are now available that provide protection against FeLV and its related diseases.

Feline Herpesvirus

Feline herpesvirus I may result in abortion during the fifth or sixth week of gestation. Lesions may be found within the uterus; however, placental lesions have only been demonstrated after experimental infection. Classically, the organism causes feline viral rhinotracheitis, and in the naturally occurring disease, abortions are thought to be the result of a nonspecific reaction to the infection. Transmission of the virus occurs via the respiratory tract, with up to 80% of cats remaining as chronic carriers. The diagnosis of herpesvirus infection is based upon the clinical signs and the isolation of the virus. Vaccination of queens provides good immunity and should be recommended for all breeding animals; however, vaccination does not prevent development of the carrier state, in which stress may result in a return of viral shedding.

Feline Panleukopenia Virus

Feline panleukopenia virus is transmitted by direct contact with saliva, faeces, and urine. Infection of pregnant queens may result in abortion, stillbirths, neonatal deaths, and fetal cerebellar hypoplasia. These effects are the result of transplacental infection, leading to fetal death and resorption in early pregnancy, and cerebellar hypoplasia**,** when infection occurs from the middle third of pregnancy onwards. Diagnosis may be made on the basis of the clinical signs, histopathological findings, virus isolation, and paired serum samples that demonstrate a rising antibody titre. There is no treatment for kittens with cerebellar hypoplasia.

Feline Infectious Peritonitis Virus

Feline infectious peritonitis virus has been implicated as a cause of infertility, stillbirths, endometritis, resorption and abortion, chronic upper respiratory tract disease, and fading kitten syndrome. Queens are not always ill and may suffer resorption, which is unnoticed. Abortion generally occurs during the last 2 weeks of pregnancy. Diagnosis is made by serological and pathological investigation.

Toxoplasma Gondii

Toxoplasma gondii infection has been incriminated as a rare cause of abortion and congenital infection of cats. It is thought that pregnancy loss is caused by systemic illness in the dam rather than induction of uterine or fetal lesions. Transplacental infection, or transmission by suckling, presumably explains how *T. gondii* cysts have been identified in very young animals before the likely time for establishing cysts via ingestion of the organism after birth. Serological screening is necessary to demonstrate the role of this protozoon in cases of abortion. Raw or undercooked meat should not be fed to seronegative pregnant queens.

Chlamydophila Felis

There is limited evidence that *Chlamydophila felis* causes abortion in the queen. The mode of transmission has not been elucidated, although the organism has been isolated from the genital tract of infected cats, and there is circumstantial evidence associating infection with reproductive disease. As well as direct isolation of the organism, diagnosis is possible by demonstrating high antibody titres. It is difficult to confirm whether the isolation of this organism indicates its role in an abortion because it may simply be an opportunistic bacterium. Vaccination is highly protective and, in most cases, results in maternally derived antibodies that persist until approximately 2 months of age.

Cystic Endometrial Hyperplasia and Pyometra

Aetiology

Both naturally occurring and experimentally induced cystic endometrial hyperplasia and pyometra have been described in the queen. The syndrome is more common in older animals and is uncommon during winter when queens are generally acyclic. In naturally occurring cases, CL are present within the ovaries, suggesting that progesterone is involved in the pathogenesis in a similar manner to the bitch. Interestingly, approximately half of the cases are seen in unmated queens, in which there should be no luteal phase. This may be explained by the observation of Lawler et al (1993),

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which showed that 35% of queens ovulated spontaneously without copulation or mechanical stimulation of the cervix.

The bacteria isolated from cases of pyometra are similar to those found in the bitch, frequently being opportunistic organisms that are normal inhabitants of the genital tract of cats, including *E. coli.* Culture of vaginal swabs is therefore of limited value.

Clinical Signs and Diagnostic Features

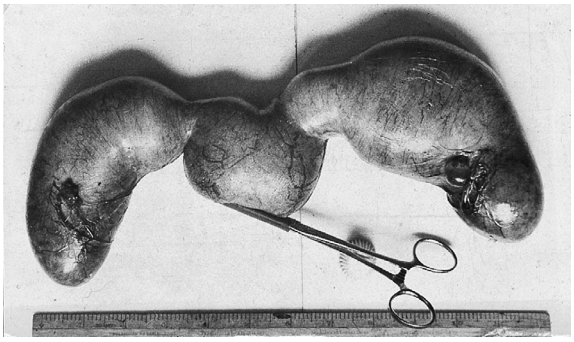
The severity and clinical signs of the cystic endometrial hyperplasia/pyometra complex vary considerably. In early cases, there may simply be mild endometrial hyperplasia with glandular dilatation. This leads to a hostile uterine environment, which may lead to fetal resorption. Queens with endometrial hyperplasia are often clinically normal, although occasionally the uterus may be enlarged and can be palpated. Ultrasound examination of these cases can reveal the presence of anechoic regions within the uterine mucosa, representing cystic glandular tissue; occasionally, free uterine fluid may be detected.

Cases of pyometra are not often difficult to diagnose because many queens have a malodorous vulvar discharge, although some queens may be particularly fastidious and regularly clean their perineum. Often, oestrus will have been observed within 2 months of the onset of the discharge. The range of diagnostic methods described in the bitch may be helpful to confirm the diagnosis. The use of real-time diagnostic ultrasound provides the most accurate method, although radiography, haematology, and clinical examination are also rewarding. Care must be exercised in palpating the abdomen because of the risk of causing rupture of the uterus.

Treatment

As in the bitch, ovariohysterectomy is the treatment of choice for pyometra. Attention should always be paid to the electrolyte and acid–base status of the animal before surgery; intravenous fluid therapy is always warranted. The complete reproductive tract from a queen with pyometra after ovariohysterectomy is shown in Fig. 33.21.

Medical therapy may be useful and has been shown to have a good success rates using either cabergoline (a prolactin inhibitor), prostaglandins, or aglepristone (a progesterone receptor antagonist) or combinations of these preparations (Hollinshead & Krekeler 2016); these are used together with appropriate fluid and antimicrobial therapy.



• **Fig. 33.21** Reproductive tract of a queen with pyometra.

Management Factors Affecting Fertility of the Queen

In the queen, modern breeding protocols may hamper reproductive performance because of a misunderstanding of the normal reproductive physiology.

Ovulation is induced by an adequate surge of plasma LH released after coitus. In one study, single copulations induced an LH surge sufficient to cause ovulations in only 50% of queens (Concannon et al. 1980). When 4 to 12 unrestricted matings were allowed in a 4-hour period, the mean concentrations of LH were three to six times higher than for single matings, and all queens ovulated (Concannon et al. 1980). Matings at limited, predetermined intervals on single or sequential days of oestrus can result in LH release of variable incidence, magnitude, and duration. It can be seen, therefore, that restricted mating regimens, which are common breeding practice, may result in failure of ovulation in a high proportion of queens. Multiple matings should result in ovulation, although Glover et al (1985) suggested that repeated matings very early in oestrus may not result in adequate LH release, whereas similar matings in midoestrus are likely to be successful at inducing ovulation. Therefore it is important that not only multiple matings but also normal courtship be allowed to ensure that matings occur throughout oestrus and not simply at the time the breeder considers to be correct.

It is difficult to identify accurately the stages of prooestrus or oestrus in the queen; however, unlike in the bitch, the behavioural events are more repeatable. During the 1 to 2 days of prooestrus, the queen refuses copulation but is more active and may show interest in the male; this period can only truly be identified in the presence of a male. Oestrus may last between 3 and 20 days, with an average of 8 days. During this time the queen displays a crouching and lordotic stance, which facilitates mounting by the male. This response can be elicited by firmly grasping the queen by the skin on the back of the neck. Mating should be attempted commencing at midoestrus, 3 to 4 days after the initial signs of prooestrus.

Vulvar swelling during oestrus does not occur; therefore clinical assessments of the queen are of little value for determining the optimum breeding time. A small amount of white vulvar discharge may occasionally be noticed during oestrus. The examination of exfoliative vaginal cytology is useful in the queen for determining the stage of the cycle; however, the technique does not enable the prediction of the onset of oestrus because up to one-third of queens may show signs of oestrus before the vaginal smear contains evidence of cornified cells. The technique is therefore most useful for verifying oestrus. The smear may be collected either using a moistened cotton swab or by irrigation with an eyedropper containing sterile saline. Staining of the epithelial cells can be achieved using a variety of stains, including a modified Wright–Giemsa stain. Erythrocytes are not found within the vaginal smear because uterine diapedesis is not a feature of oestrus in the queen. The changes in the vaginal smear are therefore limited to changes in morphology of epithelial cells because polymorphonuclear leucocytes are also usually absent except during early metoestrus and pregnancy. The percentage of epithelial cells that are cornified in appearance changes in a similar manner to that seen in the bitch. During oestrus, more than 80% of cells are cornified, although many remain nucleated, unlike the situation in the bitch. If the queen does not ovulate, the exfoliative cells return to a state similar to that observed during anoestrus or early prooestrus. Early metoestrus is characterised by

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increasing numbers of parabasal and small intermediate epithelial cells, although debris, mucus, and polymorphonuclear leucocytes also become evident.

Care should always be taken when collecting vaginal epithelial cells because the technique may induce ovulation.

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Pharmacological Control of Reproduction in the Dog and Cat

GARY C. W. ENGLAND

Knowledge of reproductive function and disease in dogs and cats has increased over recent years. The wider availability of pharmacological preparations has improved management of reproduction in both females and males (see Chapter 8).

This chapter details common methods of prevention of breeding, describes the use of pharmacological preparations for the management and treatment of common reproductive diseases, and outlines methods for the testing of reproductive function. The reader is directed to other texts that describe adverse effects of exogenous drugs by pharmacological category (England 1998a, Verstegen 1998).

Female

Prevention of Breeding

Prevention of Oestrus

Until recently, the most common method for control of breeding has been the administration of progestogens to bitches or queens when in anoestrus. This appears to alter gonadotrophin secretion and therefore prevents a return to cyclical activity (Beijerink et al. 2008). Progestogens are given either orally daily at low doses for up to 2 years or are depot preparations given by subcutaneous administration that may be repeated at 4 to 6 monthly intervals. Generally, most females will cycle normally when therapy stops, although the return to oestrus is not immediate after withdrawal of the progestogen; at least in the bitch, it appears that there is a period of inactivity similar to that which occurs physiologically, namely anoestrus, after a normal luteal phase. Progestogens are not recommended in breeding animals because of a potential effect on fertility, probably mediated by uterine changes (progestogens may induce cystic endometrial hyperplasia and pyometra). A similar effect of prevention of oestrus can be achieved by the administration of androgens (e.g., depot or daily testosterone esters), but such use is not widespread.

More recently, a long acting gonadotrophin releasing hormone (GnRH) agonist implant, deslorelin, has been used to prevent oestrus in both bitches and queens (see Lucas 2014). Insertion of the implant produces an initial release of FSH and LH which is commonly associated with signs of oestrus and can be fertile (in fact removal of the implant at this time can be used as a method for oestrous induction; see later), but there is then receptor downregulation and induction of sexual inactivity. The duration of effect relates to the dose of drug present in the implant but can be 12 months or greater. Fertility after the return to cyclical activity is good.

Although not available in all countries, there has been recent interest in the GnRH antagonist acycline (see Gobello 2012). This drug causes a decrease in FSH and LH concentrations and appears promising for future development to prevent oestrus in dogs and cats.

Queens are seasonal, long day breeders, in which hypothalamic function is downregulated by melatonin produced when days are short. Melatonin implants are commercially available for stimulation of hypothalamic function in ewes (which are short day breeders in which melatonin stimulates a return to oestrus), and administration of these melatonin implants to anoestrus cats will prevent the return to oestrus potentially for several months (Faya et al. 2011). They may also be useful within the breeding season, although the duration of effect is shorter.

Suppression of Oestrus

When prooestrus or oestrus has already begun, it is possible to inhibit the progression of the cycle by administration of progestogens similar to when used for prevention of oestrus, although higher doses are commonly used. Anecdotally, there is greater risk of inducing pyometra with this regime because progestogens are given to the oestrogen-primed tract. For this reason, some progestogens that are more potent stimulants of endometrial development are not licensed for suppression of oestrus (e.g., medroxyprogesterone acetate). Androgens tend not to be efficacious for suppression of oestrus that has already begun. Although the gonadotrophin releasing hormone agonist deslorelin is efficacious, the downregulation takes some time, and it is possible that the bitch may be mated and become pregnant at that oestrus. (In effect, the drug does not cause suppression of oestrus, but as long as the bitch is not mated, the treatment can be administered during oestrus and will prevent the return to a subsequent oestrus.)

Treatment of Unwanted Mating

Historically, the most common method for treatment of an unwanted mating in the bitch was the administration of oestrogens during a short window after mating, whereas in the queen, a similar regime using a high dose, short-acting progestogen was recommended. These regimes appear to interfere with the functioning of the uterine tube and are relatively efficacious at creating

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a hostile environment that prevented the establishment of pregnancy. Both regimes, however, risked induction of pyometra (see Chapter 33). More recently, a progesterone receptor antagonist, aglepristone, has become available, which has a mean residence time of approximately 6 days, when given at two doses 24 hours apart (see Gogny & Fieni 2016). Aglepristone can be used around the time of mating (including from shortly before ovulation) to prevent the establishment of pregnancy. This preparation can also be used at any time after ovulation to induce embryonic resorption or fetal abortion (see next section).

Termination of Pregnancy

As noted previously, progesterone-receptor antagonists such as aglepristone can be administered in the first one-third of pregnancy to induce embryonic resorption. If used early, there may be no signs of the resorption, although a coloured vulvar discharge is more common the later in pregnancy that treatment commences. In mid- and later pregnancy, the outcome of termination is abortion. The efficacy of pregnancy termination appears to be reduced when aglepristone is used alone in later pregnancy, and a more successful outcome occurs if treatment also includes prostaglandin (which causes uterine contraction and aids in expulsion of the fetal material). Prostaglandins may be used alone for this purpose as long as treatment commences more than 25 days after ovulation (the early corpora lutea (CL) do not respond well to exogenous prostaglandin and require higher doses than are required later in the luteal phase), and treatment is given daily over at least 5 days. When administered in this manner, prostaglandins cause lysis of the CL, progesterone concentrations decline, and the pregnancy is lost. Disruption of the pregnancy and expulsion is aided by the spasmogenic action of prostaglandin.

Prolactin inhibitors such as cabergoline can be used to end the luteal phase and terminate pregnancy because prolactin is the most significant luteotrophic factor in the bitch and pregnant queen (Onclin et al. 1993). Administration of daily cabergoline from day 25 after ovulation onwards results in loss of luteotrophic support for the CL, and so progesterone concentrations decline, and the pregnancy is lost. Early administration results in resorption and later administration abortion. In practice, prolactin inhibitors are normally given in a combination regime with prostaglandins because this improves efficacy as the prostaglandin acts to lyse the CL and cause uterine contractions that help disrupt the pregnancy.

Managing Reproduction

Induction of Oestrus

As a seasonal breeder, most queens will return to cyclical activity if they are exposed to more than 14 hours of light per day.

In the bitch and queen with primary anoestrus (delayed puberty) or secondary anoestrus (failure to return to cyclical activity), it is possible to induce a fertile oestrus by administration of the GnRH agonist deslorelin (Kutzler et al. 2009). The initial response to inserting a deslorelin implant is the release of FSH and LH, which causes follicle growth and results in a return to oestrus. At this point the implant needs to be removed to prevent later receptor downregulation. Fertility with deslorelin-induced cycles appears good.

More complex regimes using exogenous equine chorionic gonadotrophin (eCG, which is FSH-like in activity) and human chorionic gonadotrophin (hCG, which is LH-like in activity) are not particularly efficacious and risk causing overstimulation and ovarian disease.

Prostaglandins are not efficacious for the induction of oestrus in the bitch because. although if administered during the luteal phase, they cause lysis of the CL, and the bitch then enters anoestrus. Interestingly, through an uncertain mechanism, the administration of prolactin inhibitors (e.g., cabergoline) can be used to induce oestrus in the bitch (Spattini et al. 2007). Clearly, as a prolactin inhibitor, cabergoline would shorten the luteal phase if given at this stage of the cycle, but continued administration also shortens the subsequent period of anoestrus. If treatment commences in anoestrus, then oestrus may return relatively quickly (between 2 and 6 weeks of daily treatment), and pregnancy rates are high (greater than 80%), whereas longer treatment is required and pregnancy rates are lower (approximately 60%) if treatment commences when the bitch is in the luteal phase. Prolactin inhibitors are not efficacious for induction of oestrus in the queen.

Stimulation of Ovulation

Stimulation of ovulation may be attempted in several clinical situations: (1) in the bitch in attempt to bring forward the day on which ovulation is going to occur (hastening ovulation which may be required to assist with an artificial insemination regime, for example); (2) in the bitch if oestrus has been prolonged because ovulation has not occurred; (3) in the queen when there is an inadequate mating regime such that ovulation is not stimulated by mating; (4) in the queen as part of a regime to test whether there has been incomplete ovariectomy, and there is retained ovarian tissue; and (5) very rarely in either species when follicle cysts have been identified.

What is clear is that in all circumstances to stimulate ovulation, there must be follicles present that are in a physiological state that will allow them to ovulate. (It is not possible to stimulate ovulation in an anoestrus female; first oestrus must be induced.)

A simple method for the stimulation of ovulation is the administration of hCG, which is LH-like in activity. Single or daily administration of hCG may be required based upon the clinical circumstances. A similar effect can be achieved in both species by the administration of short-acting GnRH agonists – for example, buserelin – that results in the endogenous release of LH and FSH.

Stimulation of Milk Production

Bitches and queens occasionally have inadequate milk production, usually seen in primigravida, or associated with a Caesarean operation performed before term, or, occasionally, in bitches with poor body condition or concurrent disease.

In these cases, there will be poor development of the mammary glands, and this clinical feature can be used to differentiate the condition from failure of milk let down (see next section). In both species the repeated twice daily administration of metoclopramide can be useful to increase prolactin concentrations and thus stimulate milk production. A clinical effect can be seen within approximately 4 days. Neonates should be encouraged to suck during this period, but, of course, they will need supplementary feeding.

Stimulation of Milk Let Down

Cases of inadequate milk let down are occasionally seen in nervous primigravida and may be associated with inadequate oxytocin production. Usually, the female has mammary gland development, but attempts to express milk fail. In these cases, treatment with a single low dose of oxytocin is usually effective in allowing milk to be released almost immediately.

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Treatment of Reproductive Disease

Several aspects of reproductive disease are detailed within the previous section on managing reproduction, including cases of failure to cycle and prolonged oestrus. In this section of the chapter, consideration is given to the treatment of other reproductive disease; the headings are presented in chronological order (relating to the luteal phase, pregnancy, and postpartum).

Treatment of Pseudopregnancy

In the bitch, pseudopregnancy is common and normal in all non-pregnant females. In many cases the clinical signs are minor and are overlooked by the owner, whereas in some, they are significant and serious, particularly if they result in aggressive behaviour or mastitis. Pseudopregnancy is caused by the normal increase in plasma prolactin concentrations that commence approximately 3 weeks after ovulation. Not all cases of pseudopregnancy require treatment, but in those that do, administration of progestogens causes a rapid decline in prolactin concentrations. Although a variety of progestogen regimes are efficacious and have been widely used, there are adverse effects of the progestogens at this stage of the cycle, including the risk of pyometra. It is imperative that bitches are confirmed to be pseudopregnant before treatment with progestogens. If they are pregnant (owners may be unaware of this), progestogen treatment can delay or prevent parturition, resulting in significant illness in bitches (England 1998b).

The daily administration of the prolactin inhibitor cabergoline has been used for the treatment of pseudopregnancy (Harvey et al. 1999). Shortly after commencement of treatment, prolactin concentrations decline, and both milk production and behavioural changes are reduced. Treatment for 4 to 6 days is efficacious in nearly all cases; those that have not fully resolved usually require extension of the treatment period to a total of 7 to 10 days. Inadvertent treatment of a pregnant bitch with cabergoline will result in pregnancy termination.

Cabergoline is also effective for the treatment of chronic pseudopregnancy (commonly seen as an iatrogenic problem caused by surgical neutering, by ovariectomy, during the luteal phase); here, an extended treatment period of 3 weeks using a dose-reducing regime is usually warranted.

Treatment of Pyometra

Pyometra in the bitch and queen is a condition of the luteal phase and will have been initiated when progesterone concentrations are elevated. Progesterone drives the clinical condition by closing the cervix, increasing uterine secretions, and reducing uterine contractions. The mainstay of treatment has always been surgical removal of the uterus and ovaries, and this still remains the case because recurrence after medical treatment is not uncommon.

The principle aim of medical treatment is to remove the stimulatory effect of progesterone. Prostaglandins can be extremely effective for the medical treatment of pyometra, in which repeated low dose therapy will cause lysis of the CL, resulting in cessation of progesterone secretion. In addition, prostaglandins have a uterine spasmogenic effect, and this can aid in the expulsion of uterine pus. However, stimulating uterine contractions can be dangerous if the cervix is not open (so called 'closed cervix pyometra'), as this may cause uterine rupture. The prolactin inhibitor cabergoline is also useful in the medical treatment of pyometra because it removes the support for the CL, and, as a result, progesterone concentrations decline. Prolactin inhibitors have no direct uterine spasmogenic action; therefore a common clinical regime is their combined use with a prostaglandin (England et al. 2007). The prolactin inhibitor is normally given for several days before the administration of prostaglandin; as a consequence, progesterone concentrations will have already declined, and the cervix will have opened before the prostaglandin is given.

Attempts to treat pyometra have also been made using progesterone receptor antagonists such as aglepristone (Contri et al. 2015). These drugs block the action of progesterone but are probably best used in a regime with prostaglandin to encourage evacuation of uterine contents.

A small case series recently documented the potential for treatment of pyometra using the GnRH antagonist acycline, which appeared to be principally mediated by its action blocking progesterone secretion (Gobello 2012).

Habitual Abortion

There is debate about whether low progesterone production from the CL, in the presence of an otherwise normal pregnancy, is a true clinical syndrome in the dog or cat. Most cases of pregnancy loss appear to be associated with an abnormal uterine environment, abnormal pregnancy, or due to infectious disease. It is true that females that have recently suffered resorption or abortion will have low plasma progesterone concentrations; however, this is likely to be a result of the pregnancy loss rather than the cause of the pregnancy loss. It is only in the German shepherd dog and related breeds that a syndrome of ovulation failure or short luteal phase has been documented (Gunzel-Apel et al. 2006). Females with this problem appear to suffer from a recurrence at subsequent pregnancies; so once the problem has been documented, it is possible to try to encourage ovulation with exogenous hCG (as described previously) or to consider supplementation with exogenous progesterone or progestogens after ovulation. In the latter situation, although maintenance of the pregnancy is possible, care should be taken to ensure the lowest possible dose of progestogen is used because this commonly causes reproductive tract abnormalities in the developing fetuses.

Treatment of Uterine Inertia

Primary uterine inertia is the most common cause of dystocia in the bitch and queen. Predisposing factors appear to be a large or a small litter size, or a dam that is obese, or debilitated by age or concurrent disease. In many cases, it is feasible to stimulate uterine contractions by the administration of exogenous oxytocin; however, care should be taken before implementing this treatment especially if the litter size is small (because fetuses may be large, and obstructive dystocia may result) or if the litter size is large (because multiple doses of oxytocin are often required, resulting in a prolonged parturition). Furthermore, attention must be paid to the dose of oxytocin administered; commonly, dose rates used are too high and result in a prolonged tetanic uterine contraction rather than coordinated expulsive contractions. In most cases, a dose of 0.04 IU/kg is appropriate, given every 30 minutes for three doses. Note that oxytocin administration will cause placental compression and worsen any fetal hypoxia, so may it be contraindicated if fetuses are bradycardic. Concurrent calcium administration will often help improve uterine contractions; a common regime is 1.0 mL/kg of a 2.5% calcium gluconate solution administered subcutaneously.

Retained Fetal Membranes

Retained fetal membranes are uncommon in both the postpartum bitch and queen but are often suspected because expulsion of a placenta does not always follow each fetus. Confirmation of retained

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fetal membranes can be made on ultrasound examination. In the immediate postpartum period, two or three doses of oxytocin at 0.04 IU/kg given every 30 minutes can induce expulsion; however, the number of uterine oxytocin receptors decreases quickly after parturition, and by 48 hours, there is minimal clinical response to exogenous oxytocin. Cases presented later than this time period are best managed as for metritis (see next section).

Postpartum Metritis

Metritis in the bitch and queen occurs when, after contamination of the uterus at parturition, bacteria invade uterine tissue usually at the sites of previous placental attachment. The condition is uncommon but is more likely after dystocia and placental retention. Metritis may occur rapidly in the postpartum period, but cases may not be presented for veterinary examination until 2 or 3 days postpartum; at this time, there is likely to be limited clinical response to oxytocin because of the decrease in the number of uterine oxytocin receptors. Because metritis occurs postpartum, plasma concentrations of progesterone are basal, and so progesterone receptor antagonists are ineffective. Low dose prostaglandins may, however, be useful for their uterine spasmogenic effects (rather than their luteolytic effects), which can aid expulsion of uterine fluid. Prostaglandins are metabolised quickly, and so treatment is required two or three times per day.

Testing Reproductive Function

Testing for Ovarian Tissue

Incomplete removal of ovarian tissue at the time of ovariohysterectomy for neutering is not uncommon in veterinary practice, and although in most cases it is clear that the animal has returned to oestrus, there are a number other conditions that may have similar presenting signs. These include: nonoestrous mounting behaviour, nonoestrous vulval discharge, sexual interest from males, and iatrogenic pseudopregnancy. For these reasons, it is not uncommon to consider the need for testing for the presence of ovarian tissue (England & White 2016).

In the bitch and queen, oestrous behaviour and the physical changes associated with oestrus may be sufficiently diagnostic. However, it is also relatively simple in the bitch to measure plasma progesterone in the 65 days after alleged oestrus. In the case of incomplete ovariectomy, progesterone concentrations will be elevated (> 2.0 ng/mL / 6.5 nmol/l), whereas values will be basal in the animal that has been completely ovariectomised. Because the queen is an induced ovulator, progesterone will not be elevated unless she has been induced to ovulate either as a result of mating, vaginal stimulation, or administration of hCG (see stimulation of ovulation previously discussed). Progesterone can be measured after either of these events as described in the bitch.

For the bitch and queen, measurement of basal oestrogen concentrations at the time of alleged oestrous behaviour is often not useful in the investigation of cases of presumed incomplete ovariectomy. It is, however, possible in both species to stimulate the release of oestrogen by the administration of hCG (which is LH-like in activity) or short-acting GnRH (e.g., buserelin, which stimulates an endogenous rise in LH) when the female is remote from a spontaneous oestrus. These stimulation tests are very effective in bitches that are in anoestrus or the luteal phase but are not effective when the bitch is in prooestrus or oestrus (when basal oestrogen is, or has just been, elevated). For either test, a blood sample is collected before drug administration (44 IU/kg hCG or 2.0 μg/kg GnRH intramuscularly) and repeated 2 hours later, when a two to three times increase in oestrogen concentration is diagnostic of incomplete ovariectomy.

It is also possible in the bitch and queen to measure plasma anti-Müllerian hormone, which is basal in animals with no ovaries because it is produced by granulosa cells. Both FSH and LH are elevated in animals with no ovaries, but measurement of these hormones is not feasible in clinical practice.

Male

Prevention of Breeding

Interestingly, several aspects of male behaviour are learnt and reinforced by repetition, and so although hormonal treatment may prevent a fertile mating, it may not always abolish breeding behaviour. Indeed even castrated males, particularly dogs, may continue to achieve erection and mate.

Contraception

There has always been interest in medical contraception in dogs and cats, commonly as a temporary method to prevent fertile breeding. Until recently, the most common methods have been administration of either depot progestogens or daily oral progestogen therapy (England 1997). Similar to the bitch, the use of these regimes in the male reduce FSH and LH concentrations, with a resultant inhibition of spermatogenesis and steroidogenesis. Semen quality and plasma testosterone concentrations decline quite rapidly and remain suppressed throughout the duration of treatment. It is not always the case that the male produces no fertile sperm, in which case contraception may not be complete. There are no studies on when there is a return to fertility or detail of any long-term effects. A similar reduction in FSH and LH can be achieved by administration of exogenous androgens or oestrogens, and either could be used as contraceptive agents; this is not practised, however, because the use of androgens would result in continued male behaviour, and oestrogens have significant potential adverse effects.

Recently the depot GnRH agonist implant deslorelin has been examined in clinical studies in both dogs and cats (Lucas 2014). Insertion of the implant causes an initial stimulation of FSH and LH release with few clinical manifestations of this (unlike the return to oestrus seen in the female), followed by receptor downregulation, and a rapid reduction of spermatogenesis and steroidogenesis. Testes decrease in size, semen quality deteriorates, and male behaviour is reduced. The duration of the effect is related to the length of action of the implant.

The GnRH antagonist acycline has been shown to impair spermatogenesis over a short period of time. This drug may offer an interesting opportunity as a contraceptive for the future.

Managing Reproduction

Unwanted or Antisocial Behaviour

Much normal behaviour demonstrated by entire male animals may be inconvenient or unwanted by the owner. The methods described previously for inducing contraception by impairing spermatogenesis and steroidogenesis may be useful therefore for controlling unwanted male behaviour (e.g., roaming, mounting, urine marking). Some of these behaviours may be learnt and can persist even after castration (for example, urine spraying in tomcats). In these instances, methods that reduce plasma testosterone alone are unlikely to have any beneficial effect because in the castrated

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male testosterone will be basal. The progestogens do, however, have additional effects in that they are also sedative, and they may therefore modify unwanted male behaviour that persists in castrated animals.

Poor Libido

In most cases, poor libido is associated with the experience of a male, the mating environment, his preference for particular females, and the dominance of the female. It is rare for there to be a pathological reduction of libido without testicular disease (i.e., the hypogondal male will have small testes, low testosterone production, and poor libido). Nevertheless, owners may request hormonal stimulation of libido. What is important is to recognise that, although a temporary increase in dominance and libido may be achieved by administration of androgens, their use will cause suppression of FSH and semen quality will significantly deteriorate. A short-term increase in plasma testosterone and, anecdotally, increased libido may occur after administration of hCG.

Treatment of Reproductive Disease

Prostatic Disease

Many cases of nonneoplastic prostatic disease are stimulated by androgens, such that exogenous hormone therapy, which reduces testicular androgen production, offers good short-term control of the problem. Permanent treatment can be achieved in many cases by castration, and this should be the first line of treatment that is contemplated.

In situations in which there is prostatic disease and the male is required as a breeding animal, it may be appropriate to consider medical management at least in the medium term (months). The most useful preparations are the 5α-reductase inhibitors (e.g., finasteride), which prevent the conversion of testosterone into the active dihydrotestosterone. After administration, both serum and prostatic dihydrotestosterone concentrations are significantly reduced and a significant portion of the action of testosterone blocked. There can be dramatic clinical improvements after use of finasteride, and, importantly, although ejaculate volume decreases, sperm morphology and motility are not affected, and fertility can be maintained (Iguer-Ouada & Verstegen 1997). Medium duration treatment may allow the animal to be used for breeding or for semen to be collected and preserved before, ultimately, castration is performed.

In other situations, medical management may be required in an attempt to avoid surgery, and if preservation of fertility is not important, a good clinical response can be achieved by the administration of daily oral or depot progestogens or by administration of depot GnRH agonist preparations such as deslorelin. In the latter situation, deslorelin acts as previously described in causing an initial increase in the release of LH and FSH (and thus a short-term increase in testosterone), which is followed by receptor downregulation that results in basal concentrations of testosterone.

Cryptorchidism

Treatment of cryptorchid boys, with either GnRH or hCG, has a success rate in approximately 20% of cases (Pyoral et al. 1995) when administered twice weekly for up to 4 weeks. In dogs and cats, there are no similar large scale studies demonstrating efficacy. In most cases, the condition is inherited as a sex-limited autosomal recessive trait; as such, animals are best removed from the breeding programme.

Testing Reproductive Function

Testing for Testicular Tissue

Anorchidism is very rare in dogs and cats, and most males that present with an absence of scrotal testes are either bilateral cryptorchids that have been normally castrated or, rarely, unilateral cryptorchids in which the scrotal testes have been removed. Male-like behaviour may persist after castration and is often not helpful for differentiating these cases. Although basal testosterone concentrations are higher in males with testes than those without testes, there are diurnal variations in testosterone concentration, and nadir values can be very low (0.5–1.5 ng/mL (1.7–5.2 mmol/l)). Because these are similar to those of animals with no testes (<0.5 ng/mL (<1.7 mmol/l)), it can result in a false diagnosis of the absent testicular tissue. Generally, the roughly circadian rhythm results in highest testosterone values in the morning, so sampling at this time is the optimum if a single sample is to be tested. Preferably, hCG or a GnRH analogue should be used to stimulate testosterone secretion. A basal plasma/serum sample is obtained, hCG (44 IU/kg IM) or GnRH analogue (2.0 μg/kg IM) is injected, and a second blood sample is taken 60 to 120 minutes later. In both cases, a significant increase in testosterone concentration (>5.0 ng/mL (17 mmol/l)) in the poststimulation sample is diagnostic of the presence of testicular tissue. There should be no false-positive diagnoses.

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PART 5

Male Animal

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Evaluation of the Fertility of Breeding Males

MICHAEL MCGOWAN

Overall Approach

In veterinary practice, the clinical evaluation of fertility of breeding males most commonly involves conducting a systematic breeding soundness examination. The primary objective of this examination is to improve client decision making about the selection and management of their breeding male(s). Typically, breeding males are examined before purchase or selection into a herd/flock/kennel or breeding unit, annually before commencement of the breeding season, and as part of an investigation of the cause of lower than expected reproductive performance. Competent evaluation of the fertility of a breeding male requires the veterinarian to have good knowledge and understanding of the species' husbandry management, reproductive anatomy and physiology, and diseases and abnormalities affecting reproductive function. A common misconception is that the breeding soundness examination of a male consists only of examination of the reproductive tract. This ignores the close link between overall clinical health and normal reproductive function. In particular, the testes are quite susceptible to both systemic and local insults. For example, a bull that has acute footrot (foul-in-the-foot) will have, as well as the obvious reduced mating ability, reduced gonadotrophin hormone secretion due to the pain it is experiencing and significantly increased systemic concentrations of corticosteroids inflammatory cytokines. Each of these will adversely affect spermatogenesis resulting in a sustained decrease in semen quality. Thus the breeding soundness examination of a male animal is a standard clinical examination with special emphasis on evaluation of reproductive function.

When requested to conduct a breeding soundness examination a veterinarian must provide the owner/manager/agent with a professional opinion about the likely fertility of each male. This opinion is based on the findings from assessment of each male's functional capability – i.e., its ability to:

1. Maintain itself in a clinically normal state in the environment it is being managed in, or is likely to be managed in, in the near future;
2. Locate oestrous females and normally mount and serve these females;
3. Repeatedly ejaculate sufficient number of viable, morphologically normal spermatozoa, which when deposited in the reproductive tract of oestrous females are capable of fertilising ovulated ova and initiating normal embryonic development; and
4. Sire clinically normal offspring which have a normal life expectancy and probability of being selected as breeding animals.

In other words, each of the major components of the male's contribution to conception and birth of normal offspring must be examined. Unfortunately, each of these components is essentially independent, and thus failure to examine one or more components will significantly reduce the accuracy of one's evaluation of a male's likely fertility or the prognosis given in cases of subfertility/infertility. For example, a mature bull may be clinically normal, have an anatomically normal reproductive tract, and be producing good quality semen; but be incapable of serving because, during attempted mating, its penis spirals preventing intromission. However, when the presenting clinical signs are quite severe (e.g., marked lameness, severe swelling of the scrotum or sheath) the veterinarian may determine, for example, that a sample of semen cannot or should not be collected because it will compromise the affected male's welfare.

It is important to recognise that fertility is a continuous variable describing, in the case of male fertility, the probability of a male impregnating a population of clinically normal cycling females. Thus at the time of examination a male may be found to be sterile (permanent inability to impregnate females), infertile (temporary inability to impregnate females), severely, moderately, or mildly subfertile (reduced fertility), through to being normally fertile. Performing a systematic breeding soundness examination enables the veterinarian to detect sterile, infertile, and most subfertile males, but our ability to reliably identify highly fertile males remains an ongoing challenge. Cases of male sterility are quite uncommon, with cases of infertility more common. However, the most common male fertility problem detected is subfertility. Using the bull as an example, a fertile bull is a male that can impregnate by natural service at least 60% of 50 normal, cycling, disease-free females within 3 weeks, and 90% within 6 weeks. Therefore a mildly subfertile bull might only impregnate 40% to 50% of his females in each oestrous cycle, and a severely subfertile might only impregnate less than 20% of his females. Males experiencing severe disturbances in reproductive function may initially be infertile, then as they recover, progress from being severely subfertile to mildly subfertile before becoming normally fertile again. This highlights the importance when cases of infertility or subfertility are detected of recommending at least another follow-up examination to enable a more accurate prognosis to be provided to the client.

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Commonly, the duration of one or more waves of spermatogenesis is used to guide determination of the timing of the follow-up examination. Finally, it is important that clients understand that a subfertile male will be able to sire offspring; they simply will take much longer than a fertile male to impregnate all the females mated to them.

One of the more challenging situations in which veterinarians may find themselves is conducting a breeding soundness examination on a highly valuable breeding male that has been insured against loss of use. Although the dictionary definition of fertility that is most appropriate to male animals is 'the ability to reproduce prolifically', there have been legal cases in the past that have accepted the birth of a single offspring as evidence that a male is fertile. Thus it is important that not only clients, but also relevant insurance agents and legal advisors, are well acquainted with the objectives of the breeding soundness examination of male animals and that they understand that the examination determines whether a male is likely to be fertile but cannot guarantee it is fertile.

How to Conduct a Breeding Soundness Examination

McGowan (2004) provides a concise description of the approach to conducting the breeding soundness examination of bulls. This approach can be readily adapted and applied to any species.

Practical Considerations

Ideally, the breeding soundness examination of males should be done within a few months of when they are due to be mated or sold. This is primarily to minimise the risk of any change in fertility status of the males between examination and use or sale. However, provided there are no significant changes in the clinical health or plane of nutrition of the males, then the findings of a breeding soundness examination are generally repeatable, certainly over a period of 3 to 4 months in the case of rising 2-year-old bulls. This statement does not apply to young pubertal males still undergoing sexual maturation, as their semen quality changes significantly over a period of 3 to 4 months after puberty.

It is important that appropriately designed handling facilities and well-trained assistants are available to enable the examination to be conducted safely. For example, Beggs et al. (2013) describe management of the common occupational health and safety risks of conducting breeding soundness examinations on bulls. Good handling skills and patience are required when conducting breeding soundness examinations to minimise the risk of the examined males becoming reluctant to enter the handling facilities in the future.

The breeding soundness examination is like any other clinical examination, requiring a comprehensive record of what was examined and what were the findings. Various recording systems have been developed (Fig. 35.1). It is very important that veterinarians understand that regardless of whether it is one or multiple males being examined, the information recorded for each male should be sufficient to generate an individual breeding soundness examination certificate. Therefore a key piece of data that must be recorded for every examination is the male's permanent identification (electronic ID, brand, ear tattoo, ear tag).

The components of a breeding soundness examination are:

- Collection of husbandry and health history;
- General physical examination including assessment of gait;
- Detailed examination of the external and internal genitalia;
- Observation of mating behaviour and coitus; and
- Collection and microscopical evaluation of semen.

There is no set order that these components must be done in. Generally, the examination commences with the collection of the history and distance examination of general health, body condition, and conformation. If semen is to be collected using an artificial vagina (AV), then generally this should be done before the physical examination to minimise stress adversely affecting expression of sexual behaviour. When veterinarians are investigating suspected subfertile or infertile males, particularly those that could involve an insurance claim or litigation, they should strongly consider making a photographic record of all abnormal findings (e.g., video recording) the male attempting to serve.

Components of the Examination

History

The following are key questions to ask.

Age of the Male(s)

As the majority of prepurchase breeding soundness examinations are conducted on postpubertal virgin males, it is important to obtain an accurate age of the male to determine whether the male is likely to have reached puberty and whether postpubertal maturation of spermatogenesis has been completed. For example, nutritionally well-managed young bulls are expected to reach puberty at 9 to 10 months of age, but maturation of spermatogenesis will require a further 3 to 4 months. Thus, if bulls are examined at 12 months of age, it is quite common to find a high proportion of them producing ejaculates with a high percentage of morphologically abnormal sperm. Timing of birth can also affect age of onset of puberty due to interactions between quality of nutrition, day length, and genetics. For example, ram lambs that are born early in the season and have good growth rates may become pubertal in their first autumn, whereas those that are born late or do not grow so rapidly will not become pubertal until the next year. Age of onset of puberty is a moderate to highly heritable trait, and thus, although a group of males may be similarly managed, there may be considerable variation in onset of puberty and completion of maturation of spermatogenesis. As males become older, the risk of development of problems such as degenerative joint disease increases, which may adversely affect libido, mating ability, and semen quality. Bulls and boars, in particular, can become very heavy as they age, to the extent that they can injure females during mounting. Hence, there is merit in determining that the sizes of sire and females are compatible.

Intercurrent Disease

Occurrence of recent systemic or localised disease, including lameness and gait abnormalities, as well as systemic inflammation or localised inflammation either involving the scrotal contents directly or resulting in significant pain (e.g., lameness), can adversely affect spermatogenesis, resulting in a decline in semen quality of the affected male. History questions should thus focus on occurrence of any health problems during the past 2 to 4 months, which covers approximately one to two waves of spermatogenesis. Wherever possible, details of the onset, duration, and severity of health problems should be determined.

Management

As part of collection of the history, it is very useful to observe how the breeding male(s) is handled; how it is housed, fed, and

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Bull breeding soundness examination recording sheet

Date of examination: .....

Owner/Manager: ..... Address: .....

Ear tattoo: ..... Brand: ..... Ear tag: ..... Electronic ID: .....

Breed: ..... Age: ..... Weight: .....

History (illness, injury, nutritional management, previous fertility): .....

.....

.....

Vaccinations (vaccine used/date): .....

Purpose of examination: .....

Examinations performed: Physical examination ☐ Semen evaluation ☐ Serving assessment ☐ Special diagnostic ☐

Evaluation[1]: Likely to be fertile ☐ Terminal sire only ☐ Should be re-examined ☐ Likely to be subfertile/infertile ☐

Comments:

.....

.....

.....

Signed: ..... Date: .....

Veterinary surgeon: ..... Address: .....

Physical examination: Meets recommended standards ☐ N = normal; A = abnormal

Body condition score ..... Eyes ..... Teeth/jaws ..... Thorax ..... Abdomen ..... Legs ..... Joints .....

Hooves ..... Gait ..... Scrotum ..... Testes ..... Epididymides ..... Scrotal circumference (cm) .....

Ampullae ..... Seminal vesicles ..... Sheath ..... Prepuce ..... Penis .....

Comments .....

.....

Semen evaluation: Meets recommended standards ☐

Collection method ..... Mass activity score ..... % progressively motile sperm ..... % normal sperm .....

Comments .....

.....

Serving assessment: Meets recommended standards ☐

Method of evaluation .....

Duration of observation ..... Number of mounts ..... Number of services .....

Comments .....

.....

Special diagnostic examination

Test used: .....

Findings: .....

[1] This evaluation applies to the date of the examination and no responsibility can be taken for any subsequent events that may affect the ability of the bull to breed satisfactorily or otherwise

• **Fig. 35.1** Example of the form of a report from a breeding soundness examination of a bull.

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cleaned; where it is required to serve; and how it is handled during service.

Occurrence of Changes in Management. Significant changes in management, such as relocation to a new farm/kennel/breeding unit or introduction to a new group of breeding males, can result in a significant increase in circulating cortisol that will adversely affect spermatogenesis.

Occurrence of Significant Changes in Body Weight/Body Condition. Details of the nutritional management of the male(s), including diet composition, amount fed, and growth rate, should be checked. When peripubertal males are fed to grow very rapidly (e.g., average daily weight gain of 1.5 kg per day in bulls between 6 and 12 months of age), they are at significant risk of developing degenerative joint disease with subsequent negative effects on mating ability and semen quality. Postpubertal males fed energy and protein well in excess of what is required for normal growth may become obese, with subsequent potential negative effects on semen quality (associated with scrotal insulation) and libido. However, males that lose substantial weight/condition also are at risk of significant testicular degeneration and reduced libido. In general, chronic and continuing deficiencies of protein and energy are likely to be of greater overall importance than micronutrient deficiencies, although the effects upon fertility can be severe when these occur simultaneously (Salisbury et al. 1978).

Vaccination Status. The veterinarian must check that young males have received the initial series of vaccinations to protect against infectious diseases that can cause death, illness, or infertility and that mature males have received any required booster vaccinations.

Previous Reproductive Performance

It should be ascertained whether the male has ever mated before and the circumstances of the mating. When males have been used for mating previously, details on breeding management (single sire vs multiple sire, male to female ratio, frequency of matings per week, total females mated per breeding season) and reproductive outcomes should be examined. In situations in which the breeding soundness examination is being done as part of investigation of lower than expected reproductive performance, the veterinarian must systematically determine both the males' and females' likely contributions to the problem. This will include analysis of breeding records to determine the timing of onset of a significant decline in performance and whether there were any significant changes in management, weather events, or health problems associated with this decline. Further, whenever possible, the performance of individual males mated to similar groups of females should be compared. A commonly encountered problem is that the number of matings with reproductive outcomes data for an individual male are too small to determine whether the apparent decline in performance is real or simply due to chance variation. For example, if the true conception rate in a population is 60% per oestrous cycle, and there are data available for the outcome of only 20 matings, then by chance alone the conception rate could be between 38% and 82%. Many of the causes of infertility do not manifest themselves until a considerable period of time has elapsed from the original insult, so that careful questioning of the owner, often over matters that may be considered trivial at the time of their occurrence, may be needed to elucidate such causes.

General Physical Examination

The primary objectives of this component of the breeding soundness examination are to:

- Detect abnormalities likely to significantly affect the male's reproductive function now or within a normal breeding life; and
- Accurately diagnose abnormalities to enable assessment of whether the effect on reproductive function will be temporary or permanent; also, the veterinarian must determine whether the abnormality is likely to be heritable.

A similar approach to conducting a standard clinical examination is used, commencing with a distance examination, including assessment of body condition, musculoskeletal conformation, and gait, and then continuing to an up-close examination after the animal is appropriately restrained. Males should be maintained in moderate to good body condition; they should be 'fit not fat'. Males that are to be mated to a high number of cycling females over a short period should be managed to ensure they are in good body condition before commencement of mating.

With bulls and other large animals not used to regular handling, the distance examination is best done with the animal standing freely in the race immediately before the crush/chute or in a small holding yard. The demeanour, posture, gait, and freedom from many gross physical abnormalities can be assessed. The gait of potential sires should be closely observed at both the walk and trot whenever possible as some males that appear to be able to walk normally display obvious gait abnormalities when forced to trot. With production animals in particular, males that display marked aggressive behaviour when being routinely managed through the handling facilities should be considered for culling, because temperament is moderately heritable, and the risk of safety incidents associated with handling these animals is high.

With the animal appropriately restrained, it should be systematically examined from muzzle to hindfoot on both the left and right side for the presence of any abnormalities likely to affect clinical health and reproductive function. It is important to recognise that the conformational anatomy of an animal is at least moderately heritable, and males that are to be used for producing future breeding stock should have normal anatomy. In the case of production animals, some leniency with respect to conformation can be applied if the male is to be used as a terminal sire. However, it is important for the veterinarian to carefully consider the effect of any abnormal conformation on the long-term function of the male and the health and welfare of its offspring. Normal conformation of the hindlimbs, including the hooves/feet, is particularly important in those males that will be naturally mated with groups of females or be hand mated to a large number of females. Some examples of likely heritable anatomical abnormalities encountered in bulls and other species include excessively straight hindlimbs (adversely affects mating ability and predisposes to degenerative joint disease), scissor claw, umbilical hernia, and entropion.

Whether the rectal temperature, heart rate, and respiratory rate are measured depends on the veterinarian's judgement of the usefulness of the findings. For example, when examining groups of young bulls before sale, the vital signs are generally not measured unless a bull presents showing signs of clinical illness. This is because the simple handling of bulls through a handling facility and the usual interactions between these animals can result in marked increases in the vital signs. However, in situations in which males are being examined before being insured, the vital signs should be recorded.

Although in many cases an accurate diagnosis of an abnormality can be based on visual and/or palpable findings, the use of the ancillary tests, including ultrasonography and cytology, should always be considered to enable an accurate diagnosis and prognosis to be provided to the client.

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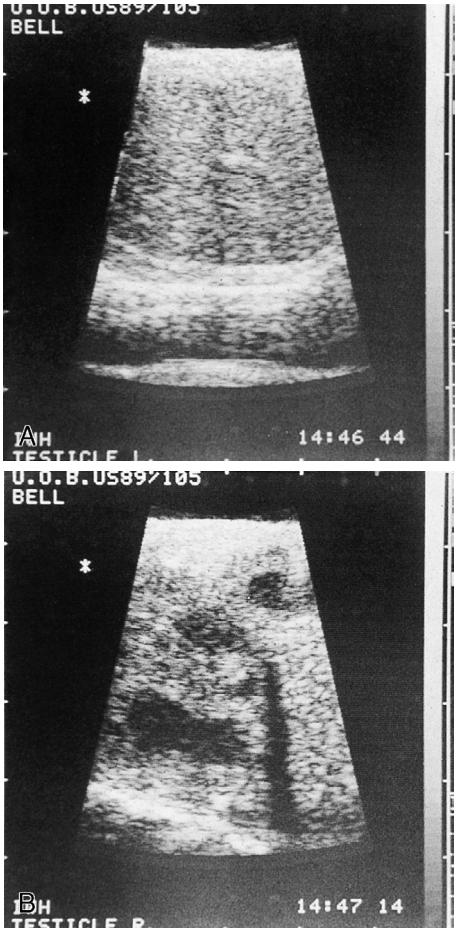
Physical Examination of the Genital Tract

The systematic physical examination of the external and internal genitalia requires that the male be appropriately restrained. How this is achieved varies depending on the species, familiarity with handling, and temperament of the male. In some cases, simple physical restraint by an assistant is sufficient, whereas in other cases sedation and/or access to purpose-built handling facilities are required. However, before the animal is restrained it is very useful to critically observe the external genitalia for any suspected abnormalities that then can be examined in more detail when the male is restrained. One of the most useful diagnostic tools the veterinarian has to either confirm or better determine the cause of palpable abnormalities of the reproductive tract is ultrasonography. Ultrasonography enables not only rapid investigation of the nature of abnormal swellings involving the testes, epididymides, penis, and accessory sex gland in particular (Fig 35.2) but also detection of abnormalities such as calcification and abscessation within the testes.

Scrotum and Scrotal Contents

Initially, the conformation of the scrotum is assessed for normal shape and symmetry and, in species such as the ram and the bull, to determine whether the length of the scrotum is abnormally short (congenital short scrotum that affects testicular thermoregulation) or excessively long (increased risk of testicular bruising, particularly if testes are large). Then the scrotum is assessed visually and by palpation for any evidence of acute or chronic inflammation and occasionally neoplasia. Trauma (lacerations), external parasite infestations (e.g., mange, ticks), and even frostbite can all cause significant inflammation of the scrotum of bulls resulting in a local 'heating' effect with subsequent varying degrees of testicular degeneration. The scrotum should feel thin ('silky') and cooler than the external inguinal region (evidence of normal testicular thermoregulation). The testes should be freely mobile within the scrotal sac, with no evidence of adhesions or fluid/material present in the vaginal space adversely affecting the veterinarian's ability to accurately palpate the testes. Next, each spermatic cord, head of epididymis, testis, and head of the epididymis is systematically palpated to determine whether they are anatomically and palpably normal. During palpation, the presence, size, texture, tone, and symmetry of each structure is assessed. To palpate the body of the epididymis requires manipulation of each testis within the sac and is often not routinely examined in production animals. Commonly encountered abnormalities of the testes include unilateral or bilateral degeneration, hypoplasia, cryptorchidism, and, in aged males of some species, neoplasia (Leydig and Sertoli cell tumours, seminoma). Orchitis is uncommon in most species, but is encountered relatively often in the ram.



• **Fig. 35.2** Ultrasonograms of (A) normal canine testes and (B) a dog with epididymitis. (With permission from Barr (1990).)

The spermatic cord, including the ductus deferens, should be palpated up to the level of the inguinal ring for the presence of abdominal contents (scrotal hernia) and down to the level of the testis for abnormalities of spermatic vasculature (varicocele), adhesions associated with trauma or ascending infection from the testis or epididymis, and vasectomy scars. The head of the epididymis should feel firm and 'meaty', whereas the tail should feel full, firm, and resilient with a smooth surface. Neither should feel swollen and 'spongy' (acute epididymitis) or enlarged, very firm, and nodular (chronic epididymitis or spermatic granuloma).

Testicular tone is a function of the firmness and resilience of each testis and provides a useful measure of the health of the seminiferous tissue. With the testis held between thumb and fingers, firm downward pressure is applied to assess firmness and then, by releasing the downward pressure, the degree of 'springiness' or resilience of the testis can be assessed. If the majority, if not all, of the seminiferous tubules, are fully functional, the testis will feel firm and resilient. However, if many of the tubules are degenerate, the testis will feel soft and 'spongy'. The tone of each testis should be assessed along its entire length, as in some cases focal areas of excessive firmness (fibrosis, calcification) or softness (focal degeneration, abscess) or alternating excessive firmness then marked softness (chronic orchitis) may be detected. Various scoring systems for testicular tone have been developed, but to be useful, the veterinarian must fully understand the descriptor for each score. A simple 1 to 5 scoring system can be used for all species as follows: 1 = very soft or fibrous feeling, both with very poor resilience; 2 = soft with poor resilience; 3 = moderate firmness and resilience; 4 = firm with good resilience; 5 = very firm with very good resilience.

Depending on the species, either measurement of scrotal circumference (for those males that have a pendulous scrotum) or measurement of the width of scrotum has been shown to be highly correlated with the weight or volume of the testes and with daily sperm output. Critically, measurement of scrotal circumference/width enables determination of whether a male has abnormal testicular development. This is particularly important when selecting males that will be used to breed future breeding stock, as testicular development is a moderate to highly heritable trait that is genetically correlated with female fertility (primarily age of onset of puberty). Measurement of scrotal circumference requires the animal to be

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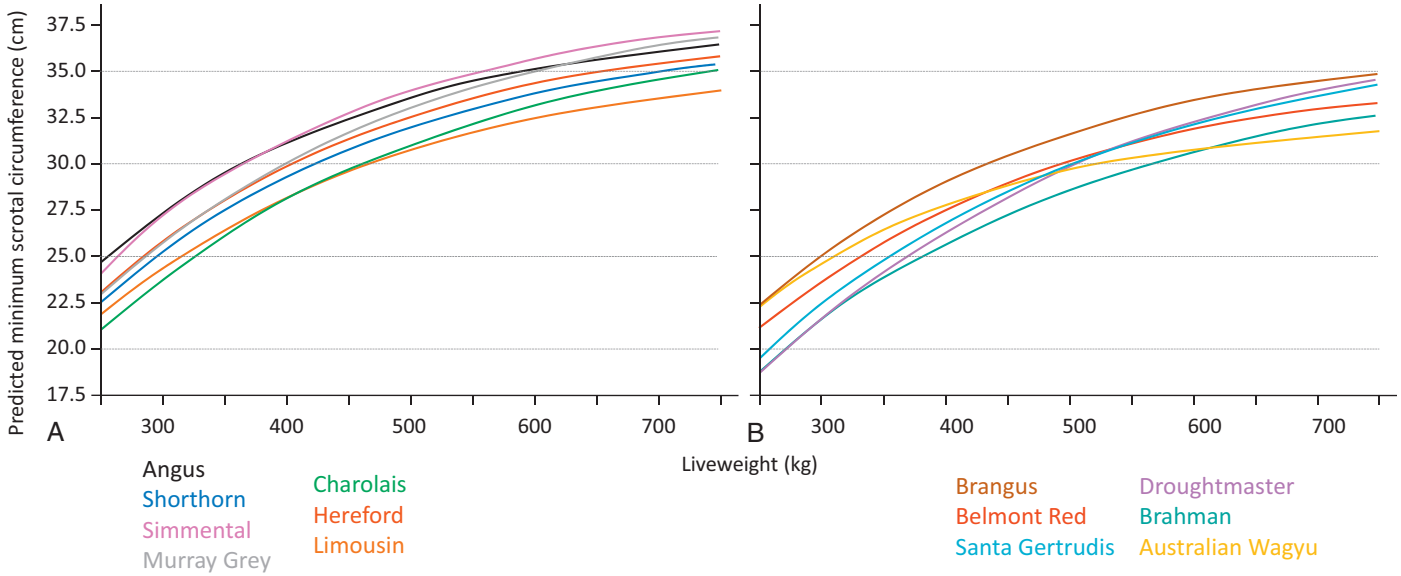
well restrained and, in the case of bulls, their hindquarters must be back firmly against a strong rail or kick gate. Both testes must be firmly massaged into the sac such that they are lying side by side, and there is no wrinkling of the scrotum. Then with the left hand clasped (with thumb pointed upwards) around the cranial aspect of the neck of the scrotum to hold both testes fully in the scrotum, the tape is looped around the broadest circumference and drawn snugly against the scrotum so that it is firmly in contact with the entire circumference. The measurement of scrotal circumference should be highly repeatable both within and between examiners. The development of scrotal tapes that enable a standard amount of tension to be applied when drawing the tape around the scrotum has greatly helped standardise this procedure (Fig. 35.3). In stallions and other males with similar scrotal anatomy, scrotal width can be readily measured either with callipers or with transscrotal ultrasound.

In young growing males, measurement of scrotal circumference/width can provide a relatively accurate guide as to whether males have reached puberty or not. For example, bulls are likely to have reached puberty when they achieve a scrotal circumference of 26 to 28 cm. Various guidelines defining the minimum scrotal circumference for young males have been published, but these must be interpreted with care as plane of nutrition, age, and breed can all strongly influence testicular development. Fordyce et al (2014) demonstrated that in young beef bulls, body weight (rate of growth) has the greatest effect on scrotal circumference. These authors defined abnormal testicular development as a scrotal circumference measurement lower than the fifth percentile at a given body weight (Fig. 35.4). When body weight data is not available, Beggs et al. (2013) have provided recommendations on minimum scrotal circumference measurements by breed and age for young bulls. A general guide is that, across breeds, if bulls have been managed on a moderate to high plane of nutrition, then at 12 months of age the scrotal circumference should be at least 30 cm, at 18 months of age at least 32 cm, and at 24 months of age at least 34 cm. Similarly, scrotal circumference of mature rams depends on body weight: values over 28 cm are acceptable for smaller breeds and over 34 cm for larger breeds. Day length can also have a significant effect in some species. The scrotal circumference of rams managed in temperate regions of the world is quite dependent on season, with a 25% to 35% increase in size between the nonbreeding and breeding season. The tone of the testes and the cauda epididymides undergo parallel changes. In young males that have passed the postpubertal peak growth phase, feeding an excessively high energy ration can result in the onset of fattening with deposition of fat in the neck of the scrotum and over the testes causing the scrotal circumference to be increased. These males are likely to have an

• **Fig. 35.3** Measurement of scrotal circumference in the bull using the regulated tension tape developed by Professor A. Barth.



• **Fig. 35.4** Minimum scrotal circumference measurement for a range of young beef breed bulls by body weight. (A) *Bos taurus* breeds. (B) *B. indicus* breeds and Australian Wagyu. (Redrawn, with permission, from Fordyce et al. 2014.)

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increased proportion of abnormal sperm in their semen due to the fat deposition adversely affecting testicular thermoregulation.

Accessory Sex Glands

The accessory sex glands are generally examined after examination of the scrotal contents. The presence, size, texture, and tone of each structure are assessed. In the bull and the stallion, the prostate, ampullae, and vesicular glands ('seminal vesicles') can be readily palpated per rectum. In the bull, pathological changes of the prostate are exceedingly rare, of the ampullae uncommon, but seminal vesiculitis is relatively common. In the stallion the ampullae are more commonly affected than the seminal vesicular glands. The bulbourethral glands, although present, are quite difficult to palpate per rectum. In large boars, rectal examination is potentially feasible, but digital examination of smaller boars will only enable palpation of the bulbourethral glands. In smaller dogs, digital examination of the prostate is possible, but radiography or ultrasonography, which is essential when prostatic disease is suspected, is required to examine the prostate of large breed dogs. Acute inflammation of the accessory sex glands manifests as pain, swelling, and loss of normal architecture on palpation, whereas nodular fibrosis, adhesions, and sometime abscessation are typical of chronic inflammation. It should be noted that bacterial infections of the accessory glands can spread to the testes and vice versa.

Penis and Prepuce

The sheath which extends from the scrotum forward to the umbilicus supporting the penis and prepuce is observed for its conformation and evidence of any abnormal swellings, trauma, or dermatitis. *Bos indicus* bulls with excessively pendulous sheaths are considered at increased risk of developing prolapse of the prepuce. The preputial orifice should be examined for any evidence of abnormal discharge or excoriation and scabbing (e.g., ulcerative posthitis in rams and bulls). In bulls, particularly, *B. indicus* genotypes, excess preputial eversion (> 10 cm everted) should be noted as there is some evidence this is associated with an increased risk of preputial prolapse. With the bull appropriately restrained, the penis can be readily palpated within the sheath and extending caudally to the base of the scrotum for any evidence of adhesions and swellings. Whenever possible, the penis and prepuce should be extruded and systematically assessed for evidence of trauma, inflammation, and neoplasia. Commonly encountered abnormalities include lacerations, superficial trauma/microbial induced inflammation, penile papillomatosis, persistent frenulum, and acute/chronic preputial prolapse. The penis and prepuce can be most commonly visually assessed and, in some cases, palpated during collection of a sample of semen. When semen is collected using an AV, the penis and prepuce can be quickly assessed immediately after removal of the AV. Generally, a high proportion of males undergoing electroejaculation will protrude the penis, allowing visual assessment and palpation if required. In bulls, simply inserting a lubricated hand into the rectum and massaging the urethralis will often result in protrusion of the penis sufficient to enable checking for abnormalities such as penile papilloma and persistent frenulum. To diagnose penile deviations requires observation of the male attempting to serve a mount animal or oestrous female. This enables assessment of whether the male is capable of achieving a normal erection, intromission, and ejaculation. Although sedating males will often result in them protruding their penis or enable manual extrusion of the penis, palpation of painful lesions may still not be possible due to the lack of desensitisation. Thus, in a small number of cases, administration of a pudendal nerve block or general anaesthesia is required to enable accurate assessment of the penis and prepuce.

Collection and Assessment of Semen

Although measurement of scrotal circumference/width and assessment of testicular tone provide a useful estimate of semen quality, only perhaps 70% to 80% of males with well developed, firm/'springy' testes produce ejaculates with a high proportion of morphologically normal sperm. This is why collection and microscopical assessment of a sample of semen should always be done as part of a breeding soundness examination of a male. The characteristics of the semen of common domestic animals are summarised in Table 35.1. The microscopical examination of a sample of semen enables the function of the testes and epididymides to be directly assessed. Details of the methods of collecting semen from the common domestic males are provided in the next sections, but regardless, it is critical that during and after collection the semen is maintained warm and away from direct sunlight. For example, when collecting semen from bulls and rams in cold conditions, it is highly advisable to have some form of insulating (woollen gloved hand, styrofoam, or felt sleeve) or warming device (modified thermos flask) around the collection tube to eliminate the risk of cold shock.

Collection From the Bull

Semen can be collected from bulls using an AV, by electroejaculation, by massage of the ampullae, or by aspiration from the vagina of a cow immediately after service. Each of these methods has advantages and disadvantages. Collection of semen by AV enables concurrent assessment of libido and mating ability, including assessment for

TABLE 35.1 Semen characteristics of domestic animals

Characteristic	Bull	Ram	Stallion	Boar	Dog
Volume (mL)	4 (2–10)	10 (0.5–2.0)	60 (30–250)	250 (125–500)	10 (2–19)
Fractionated	N	N	Y	Y	Y
Density (x10^6/mL)	1250 (600–2800)	2000 (1250–3000)	120 (30–600)	100 (25–1000)	125 (20–540)
Motile sperm (%)	>70	>90	>60	>60	>85
Normal sperm (%)	>75	>75	>60	>60	>90

Figures in parentheses indicate the normal range; *N*, no; *Y*, yes
Compiled from Arthur et al. (1989), Roberts (1986), and Morrow (1986).

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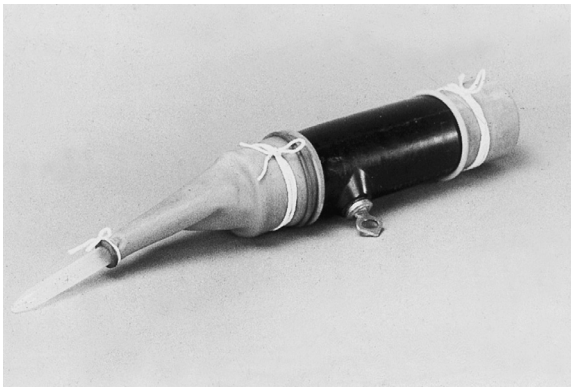

• **Fig. 35.5** Artificial vagina for use in the bull.

any evidence of penile deviation. However, it generally requires that the bull has been regularly handled or trained to serve into an AV, and the person collecting the ejaculate must be careful not to be struck by the bull when attempting to apply the AV over the penis. The AV (Fig. 35.5) consists of a strong outer rubber cylinder containing a latex liner. At one end of the AV, a latex extension cone carrying a graduated collecting tube is attached. The length of the AV should be adjusted so that the bull ejaculates into the extension cone, thereby producing semen that is as free as possible of particulate or bacterial contamination. The space between the outer cylinder and latex liner is filled with warm water, so that the temperature in the lumen of the AV is between 45°C and 48°C. If this temperature falls below about 43°C, ejaculation is unlikely. The main stimulus to ejaculation is the temperature of the AV, with pressure upon the bull's penis being relatively less important. A little inert lubricant (liquid paraffin or soft paraffin but not gynaecological jelly because this is usually spermicidal) is smeared around the lumen of the AV just before use.

Control of the bull and the safety of all personnel are of paramount importance during semen collection. A halter-trained, oestrous cow is the ideal mount animal (especially during training of a bull to serve an AV), although such an animal is often difficult to provide. A quiet non-oestrous cow or oestrogen-treated steer, at least two-thirds the weight of the bull, restrained in a purpose-built bail (same as is used for assessment of serving ability, Fig. 35.13) are commonly used as mount animals. When there is no restraint bail available, the haltered mount animal can be held in position by an assistant. Occasionally, the mount animal may need to be lightly sedated, but this needs to be done very carefully to avoid the mount animal collapsing under the weight of the bull. The bull is led to the mount animal, allowed to nuzzle and smell it, but then led away before mounting takes place. This usually causes complete or partial erection, with production of preejaculatory accessory gland secretions, and is considered to result in ejaculation of a greater total number of sperm when the bull is finally allowed to mount. The bull is then led up to the mount animal with the collector standing to the right of the offside shoulder of the bull. As the bull mounts, it makes small, preliminary thrusts with its penis to locate the vulva, and, during these, the collector grasps the sheath (not the penis) with the left hand and deflects the penis, guiding it into the AV. The bull will then normally make the ejaculatory thrust into the AV (Fig. 35.6). The entire procedure must be carried out quietly and methodically, keeping the bull under continual careful observation. It is important to note that many bulls are at their most aggressive immediately after ejaculation. When older bulls have been used for natural service for a number of years, they

• **Fig. 35.6** Semen collection from the bull by means of an artificial vagina. (With permission from Parkinson & Bruere 2007.)

may be unwilling to ejaculate into an AV. Providing the libido of the bull is sufficient, repeated teasing or allowing the bull to mount but deflecting his penis so he does not ejaculate will usually provide enough stimulation for ejaculation to occur when presented with the AV. More difficulty is experienced with bulls that are unwilling to mount in the presence of humans or that are no longer halter trained. In these cases, use of an intravaginal AV developed by Barth et al. (2004) should be considered.

A sample of semen adequate to enable microscopical assessment can be collected from around 99% of bulls using modern electroejaculation units that utilise either a three or two strip electrode intrarectal probe (Fig. 35.7) and enable sensitive control of the magnitude of electrical stimulus applied. The technique of electroejaculation involves the application of low level electrical stimulation in the form of a modified sine wave to selectively stimulate the nerves (parasympathetic outflow from the internal pudendal nerve, sacral plexus, and hypogastric nerve) responsible for erection, ejaculation, and emission. The major advantage of this procedure is that it enables collection of a sample of semen from rangeland bulls and bulls that either have not been trained to or will not serve into an AV. When required, several samples of semen can be collected over a short period using electroejaculation. In most cases the veterinarian will be able to observe the penis closely and can select to collect the sperm-rich fraction only. The major disadvantages are that the procedure does not allow assessment of mating ability and has a short-term, mild effect on the bull's welfare with stimulation of the sciatic and obturator

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• **Fig. 35.7** Electroejaculation equipment for use in the bull. (With permission from Parkinson & Bruere 2007.)

nerves causing increased hindlimb muscle contraction in some bulls. Before commencing the procedure, the veterinarian should check that the electrodes on the intrarectal probe are shiny and free of any tarnish and that the electrical stimulation unit is functioning normally. To perform this procedure, the bull must be restrained in a well-designed cattle crush/chute that provides good footing. Whenever possible, the bull's head should not be held in a head bail and but rather it should be held either behind the closed head bail or closed baulk gate, with its hindlegs back against the kick gate and its body held firmly from side to side by the sides of the crush/chute. Faeces must be evacuated from the rectum to allow intimate contact between the electrodes of the intrarectal probe and the rectal mucosa. Insertion of the probe is most commonly done after palpation of the accessory sex glands. Further, before insertion of the probe, it is recommended that the ampullae and urethralis be massaged for about 30 seconds to reduce the amount of electrical stimulation required to induce ejaculation. The probe is inserted as the veterinarian's hand is removed from the rectum. This is done to prevent air being sucked into the rectum, which adversely affects contact between the electrodes and the rectal mucosa. After inserting the lubricated intrarectal probe fully into the rectum, the tail is clamped down snugly behind the probe, and an assistant holds it in place, ensuring that the probe remains horizontal to the floor of the pelvis throughout the collection. Generally starting at the lowest setting, the bull is then rhythmically stimulated for alternating period of 2 to 3 seconds, with progressively increasing magnitude of the stimuli. It is critical that the veterinarian monitors the response of the bull to the stimulation applied at all times. Unfortunately, the response of individual bulls to the electroejaculation procedure cannot be predicted. Most bulls, as the stimulus is applied progressively, arch their back, thrust forward, commence protruding the penis, emit initially clear accessory gland fluids, and then emit sperm rich fluid. However, a small proportion of bulls are very sensitive to electrical stimuli and, even at low magnitude stimuli, show marked stiffening and/or knuckling of the hindlimbs. In these cases the veterinarian must only very slowly increase the magnitude of the stimuli to ensure the bull does not become recumbent. With some of these very sensitive bulls, the best approach is to initially apply electrical stimulation then remove the intrarectal probe and manually stimulate emission of semen.

• **Fig. 35.8** Electroejaculator for the ram.

Sequential per rectal massage of the ampullae and urethralis enables a sample of semen suitable for microscopical examination to be collected from about 80% to 90% of bulls, including yearling and older bulls. About 1 to 8 minutes of rectal massage is required. Initially, the ampullae are located and either repeatedly firmly pressed against the floor of the pelvis and then massaged in a caudal direction or grasped between thumbs and fingers and 'stripped' caudally. Massage of the ampullae is alternated with massage of the urethralis to stimulate emission of semen. This technique requires another person to collect the semen sample as it is emitted from the penis or from the preputial orifice. As emission of semen can take some time, it is important that the collection tube be kept warm. Use of this technique should be considered in bulls from whom a sample could not be collected by electroejaculation and those with either lower back problems or hindlimb lameness. The major disadvantages of this technique, apart from the 10% to 20% of bulls that yield no semen, are that the samples collected often have low sperm concentration and are contaminated with epithelial cells and debris from the preputial cavity and penis. Also, some bulls urinate immediately after ejaculation, contaminating the sample collected. Good palpation skills are required to consistently obtain semen samples by this method.

When the veterinarian wishes to collect a sample of semen from a cow after it has been served by a bull, the best approach is to infuse the vagina of the cow with a litre of saline before mating, then by rectal massage, express the saline and suspended mucous from the vagina. The semen sample collected is much easier to assess if most of the mucous has been washed from the vagina.

The Ram

Semen is relatively easy to collect from rams by electroejaculation or by the use of an AV. Unlike bulls and stallions, in which it is not uncommon to have poor semen in the absence of other abnormalities of the genital tract, it is uncommon for this to occur in the ram. Hence physical examination of the genitalia should always be undertaken in the ram before semen collection is attempted, as many infertile rams can be detected without the need for semen examination.

Most semen examinations in the field are undertaken using samples collected by electroejaculation. Some veterinarians prefer to restrain the ram in a standing position; others lay it on its side and hold the penis in an extruded position. A lubricated probe (Fig. 35.8) is placed into the rectum of the ram with the two longitudinal electrode strips faced towards the hindfeet. The probe is located on the brim of the pelvis as shown in Fig. 35.9. Ejaculation should occur after four to six rhythmic stimulations of the ampullae and sacral nerve plexus. In many rams, erection will occur if the probe

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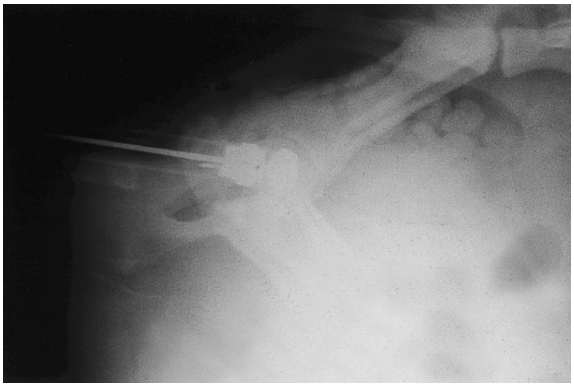
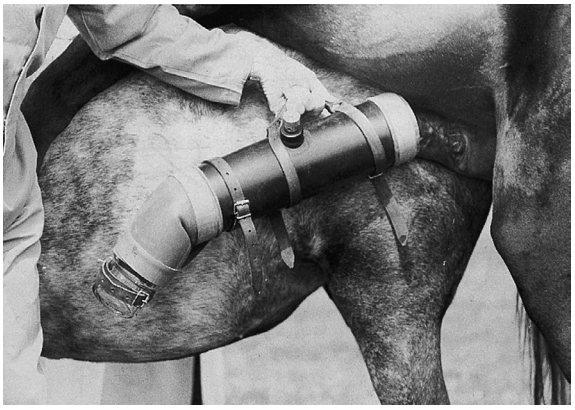


• **Fig. 35.9** Radiograph of the hindquarters of a ram indicating the position of the electroejaculator relative to the brim of the pelvis.



• **Fig. 35.10** Semen collection from the stallion. The urethra is palpated for the peristaltic waves that characterise ejaculation.

is correctly positioned, but a proportion of rams will ejaculate without an erection. Electroejaculation is generally well tolerated, but if there is widespread stimulation of hindlimb muscles or if the ram does not ejaculate after 4 to 6 stimulations, the ram should be rested for several minutes before repeating the procedure. When rams are taken directly from pasture and housed on straw for 1 to 2 days before attempting collection, the faeces become quite dry and firm, reducing contact between the electrodes and the rectal mucosa. This can reduce the chances of collecting a satisfactory quality sample. One of the problems with electroejaculation is that there is considerable variation between individuals in their response to electrical stimulation. Some produce semen samples which have similar volume and density to an AV ejaculate, whereas others have markedly reduced sperm concentration (i.e., appear less dense). In the latter case the veterinarian must determine, based on their assessment of the sample's mass activity or percentage progressively motile sperm, whether the sample collected is consistent with their assessment of scrotal circumference and testicular tone (i.e., the sample collected is or is not representative of what the ram is likely to ejaculate when mated). If there is any doubt, the veterinarian should always collect another sample.

Collection of semen from rams using an AV is essentially the same procedure as for the bull. Although rams generally require some training, it is surprising how many untrained rams will serve into an AV in situations when a ewe is restrained in a modified head bail (similar in design to that used for serving ability testing bulls) in a pen adjacent to where they are held and is initially served by an experienced ram. It appears that simply observing this mating behaviour stimulates many rams to serve into an AV provided the collector is experienced, and can quickly and correctly position the AV for the ram to serve into. The AV that is used for rams is essentially a smaller version of the bull AV, except that it is common to provide some means of keeping the ejaculation cone warm. Although sheep are seasonal breeders, semen can be collected from most rams throughout the year. Semen quality can be maintained to a considerable extent by regular ejaculation, a phenomenon that is used to maintain production of (frozen) semen in sheep AI centres. On the other hand, out of season collection of semen from rams managed on commercial farms is generally more difficult than during the breeding season.

The Stallion

Although the collection and microscopical examination of a sample of semen has traditionally been regarded as the most important aspect of breeding soundness examination of the stallion, Love (2007) states that it should be regarded as but one component of the examination. The preferred method of collecting semen from the stallion is by the use of an AV. The alternative, less satisfactory method is by examining the residual drops of semen emitted from the urethra after the stallion dismounts. Collection from the vagina of a mare is generally not possible because ejaculation occurs directly into the uterus.

The AV for a stallion is larger than that used for a bull and, for large stallions, may be quite heavy. The stallion is initially teased with a mare in full oestrus. The mount mare is often restrained with a twitch or service hobbles. After mounting the penis is deflected into the AV, and its lower surface is palpated for the presence of peristaltic ejaculatory waves (Fig. 35.10). Stallions can be quite fastidious about the temperature and pressure in the AV before they will ejaculate, and some stallions object so vehemently to even the sight of an AV that collection is impossible. Care must be exercised by the collector to avoid getting struck by the stallion's forelimbs or trapped between the forelegs of the stallion and the body of the mare.

The Boar

Semen is most easily collected from the boar by manual stimulation of the penis. AVs have been designed for the boar (Melrose & O'Hagen 1959) but are less effective than the manual method. The main stimulus to ejaculation in the boar is the locking of the corkscrew-shaped distal part of the penis into the spiral-shaped cervix of the sow. Hence, after mounting an oestrous sow or, if trained, a dummy, the penis is initially stimulated through the sheath then, when protruded, the corkscrew-shaped glans is firmly grasped with a lubricated, latex gloved hand, mimicking the cervical lock. Handling other parts of the penis should be avoided (Althouse 2007).

The ejaculate is collected into a warmed, insulated container, either directly or via a funnel in which gauze is placed to separate the gel fraction from the remainder of the ejaculate. Thrusting is vigorous before the start of ejaculation but becomes less vigorous as it begins. The boar begins to thrust again as the postsperm gel fraction is produced. The presperm fraction is not collected, and the gel fraction is retained by the gauze in order that the sperm-rich fraction contains minimal quantities of these components.

The Dog

Semen can be collected fairly readily from most dogs by digital stimulation of the penis. The presence of a teaser bitch, preferably in oestrus, facilitates the procedure. Because of the adverse effects

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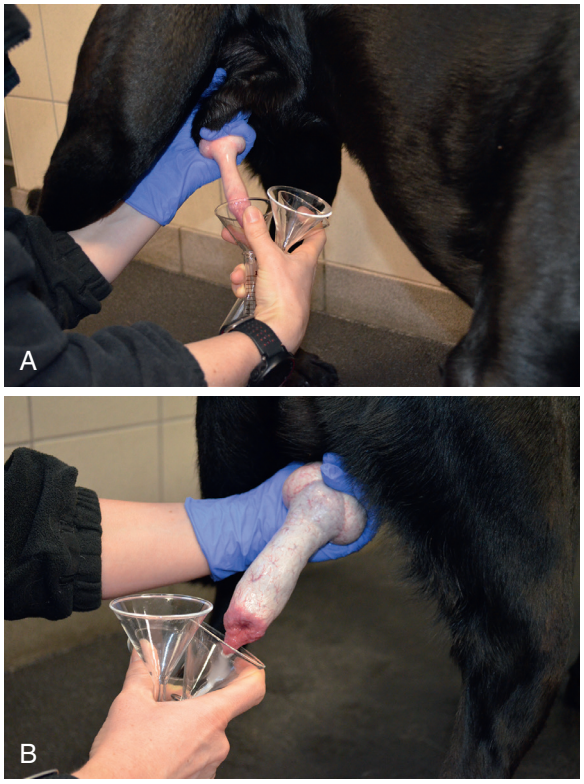

• **Fig. 35.11** Manual collection of semen from the dog. (A) The penis is grasped behind the bulbus glandis, simulating the lock of the levator vestibuli of the bitch. (B) The different fractions of semen are collected into separate collecting vessels to minimise the dilution of the sperm-rich fraction with accessory gland fluids.

of latex rubber on canine sperm, semen collection by AV is not recommended.

In order to induce ejaculation by digital manipulation, the bulbus glandis may be rhythmically compressed (Fig. 35.11), although many dogs will ejaculate simply in response to their own thrusting. Before production of the presperm accessory fluids, quite vigorous thrusting occurs, but the dog is relatively quiet during the initial phase of ejaculation. First, during this quiescent phase, between 0.5 and 5.0 mL of watery, preejaculatory fluid is produced over a period of up to 50 seconds. Ejaculation of the sperm-rich fraction then follows immediately, when 0.5 to 2.0 mL of thick, creamy fluid is emitted within a few seconds. The dog then attempts to turn and tie, whereupon the postsperm, prostatic fluid fraction of the ejaculate is produced. This third component is again watery, comprising up to 30 mL of fluid, which is ejaculated over 3 to 30 minutes.

Semen Examination

This section will focus on examination of semen and sperm as part of a breeding soundness examination and will not include examination of frozen thawed semen. The primary objective of collecting a sample of semen from a male is to evaluate the quality of sperm. It is not always necessary to collect a complete ejaculate. All that is required is a representative sample containing sufficient number of sperm to evaluate motility and morphology. This is important when collecting semen by electroejaculation as it is quite common, especially in bulls, to collect samples containing a low concentration of sperm (200×10^6/mL). The determination of whether daily sperm production is sufficient to ensure a normally expected mating outcome is generally based on measurement of scrotal circumference/width. However, when a male is to be used for an artificial breeding programme, it is not uncommon to determine sperm concentration and total sperm in the ejaculate and then to adjust these according to one's estimate of the percentage progressively motile sperm.

It is important for veterinarians to appreciate that many of the key relationships between the semen and sperm parameters and fertility have been established under ideal laboratory conditions, and thus, especially when examining semen in the field, the veterinarian must take 'the laboratory to the field' (Fig. 35.12). It is very easy to adversely affect sperm motility. Spermatozoa are very sensitive to both 'cold shock' (sudden decrease below 30°C) and heat shock (sudden increase above 40°C), changes in pH (urine contamination), and osmotic shock (contamination with water, improperly stored/prepared semen diluents). The 'golden rule' is that, at all times, whatever the semen comes in contact with must be warm, dry, and free from any dust, mould, or chemical contaminants (important to carefully rinse collection equipment after cleaning with disinfectants/soap). Use of a microscope that has either a built-in or attached warm stage and a slide warming platform (for microscope slides and pipettes), both set at 35° to 37°C, is strongly recommended. Prepared semen stains and diluents should also be maintained warm. This can be readily done by placing them in a styrofoam box containing a hot water bottle. A microscope equipped with good quality optics and preferably 4x, 10x, 40x, and 100x objective lenses is essential. A microscope that has both bright field and phase contrast capacity is preferred as the latter greatly improves assessment of the percentage progressively motile sperm. Veterinarians should practice the semen examination procedure in their clinic before attempting to conduct examinations in the field.

The ejaculate or sample of semen should be initially inspected for the presence of urine (typical smell), fresh or degenerating blood (semen will have a pink or brown appearance), pus, and dirt/faeces/smegma contamination. Some normal bulls have semen that has a lemon yellow colour due to the presence of increased levels of riboflavin in the seminal plasma. The density (sperm concentration) of the sample is often estimated based on its colour. For example, for bull semen that appears clear to slightly cloudy, it is estimated that there are less than 200×10^6 sperm/mL, cloudy to milky appearing semen 200 to 500×10^6 sperm/mL, milky appearing semen 500 to 1000×10^6 sperm/mL, and creamy appearing semen 1000 to 1500×10^6 sperm/mL. Azoospermia is a rare problem, and thus when a sample of semen is collected containing no sperm or very few sperm, the veterinarian should always attempt to collect another sample.

Assessment of Sperm Motility

The assessment of sperm motility should be done within a couple of minutes of semen collection whenever possible. In the event that semen cannot be assessed immediately, then it should be appropriately extended and stored. For those species that produce highly concentrated semen (ram, bull), a small drop of semen is often first placed on a warm slide and examined directly using the 4x lens (with the condenser turned down) for evidence of mass activity (often scored from no swirling wave to rapid swirling wave on a 1–5 or 1–10 scale). This examination enables rapid determination of whether the sample collected is consistent with the findings from examination of the scrotal contents (i.e., is representative of what the male would ejaculate if mated). However,

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it is important to note that mass activity is a function of sperm concentration and percentage motile sperm, and when samples have low sperm concentration, typical of some species and in bulls and rams due to variable response to electroejaculation, a swirl may not be observed even though the sample contains a high proportion of motile sperm. Further, observing a swirl does not allow the veterinarian to be able to always conclude that the sample contains a high proportion of progressively motile sperm, as a swirl can be observed in samples containing a high proportion of sperm swimming backwards due to the distal-midpiece reflex defect or looped tails.

To assess the percentage of progressively motile sperm, a small drop (~3 mm in diameter) of semen is placed on a prewarmed slide, and a warm coverslip is placed over it. It is critical that the droplet is small enough to ensure that a single layer of sperm can be observed. If the microscope being used only has bright field capacity, then the condenser must be turned down, the light reduced, and the 10x or 40x lens used. If the semen sample is quite concentrated, then it is advisable to dilute a drop of semen with several drops of either phosphate buffered saline or 2.9% sodium citrate solution on a warm slide, and then draw up a drop of the diluted semen and make a wet mount preparation as described previously. It is very important that the diluent is in-date and has been appropriately stored; otherwise, the pH or osmolality may have changed, and this can negatively bias the estimate of percentage progressively motile sperm. Some experience is required to accurately estimate the percentage progressively motile sperm. The veterinarian should focus on estimating the proportion of sperm that are not moving forward and initially broadly categorise the sample as having poor, fair, or good progressive motility (< 30%, 30%–59%, and ≥ 60%, respectively). The 'golden rule' when assessing sperm motility is, if in doubt, think 'there might be a problem with how I have managed the sample, conducted the evaluation or, there could be a problem with the sample itself or the bull'. Therefore, if the mass activity or percentage progressively motile sperm is inconsistent with the findings of the physical examination of the scrotal contents, examine one or more additional droplets/wet mount preparations and/or collect another sample of semen. Also, sometimes with males who are examined out of season or have not been serving, the first sample/ejaculate may contain a relatively high number of dead sperm. In these cases the motility observed in the second sample is usually much better than initially observed.

Assessment of Sperm Morphology

If a sample of semen is collected from a male, the percentage of morphologically normal sperm should always be determined. This parameter has been consistently shown to be significantly associated with mating outcomes and is almost certainly a heritable trait. It provides a sensitive measure of the health of the testes, epididymides, and accessory sex glands. Further, when males are reared and mated in environments in which animals experience seasonal extremes in temperature/humidity, plane of nutrition, and parasitism, it can provide a useful indication of how well-adapted the animal is to its environment.

The two most common methods used for assessment of sperm morphology are:

Bright-field microscopy of sperm stained with nigrosin-eosin (NE) (Swanson & Bearden 1951). NE is a 'supravital stain' that can also be used to estimate the proportion of live and dead sperm in a sample. 'Live' sperm with intact cell membranes at the time of staining do not take up the eosin and thus appear pale white against the purple (nigrosin) background. However, 'dead' sperm have damaged cell membranes and take up the eosin, with the head of the sperm appearing partially or completely pink. To make a NE stained smear, a small drop (5–6 mm in diameter) of warm NE is placed at one end of a warm microscope slide. For samples estimated to have a very high sperm concentration, simply dipping a clean, warm pipette into the sample and then into the drop of NE will pick up sufficient sperm for a smear. With more dilute samples, a drop of semen (3–5 mm in diameter) is mixed with the drop of NE. Then, either using the edge of a microscope slide or a wooden applicator stick, these are drawn up so they just touch the droplet of NE; then they are drawn slowly across the slide to create the smear. It is critical that the smear be dried quickly to avoid cracking of the stain and hypotonic shock to the sperm. After making the smear, the male's identity and date of examination must be etched or written on one end of the slide and the slide stored in a slide box. In the field, preparation of NE smears is best performed on the slide warming platform. The smear is then examined using oil immersion at 1000x magnification. Multiple fields in which the sperm are individually dispersed (i.e., not clumped on top of each other) and can be seen very clearly are selected for examination and counting of normal and abnormal sperm. Note that both 'live' and dead sperm are to be counted.

Differential-interference phase-contrast (DIC) or high-quality **phase-contrast microscopy** of cover slip preparation of preserved semen (Aalseth & Saacke 1985). Semen can be conveniently preserved using 10% buffered formal saline (BFS). Depending on the estimated concentration of sperm, one or more drops of semen are pipetted into 1.5 to 2 mL labelled, screw top, plastic containers of BFS until the fluid appears slightly cloudy. The morphology of the preserved sperm can either be immediately assessed or examined some later time. With the latter, the preserved samples should be stored at 4°C to control bacterial multiplication. Phase contrast and, in particular, DIC microscopy is considered superior to bright field microscopy for assessment of sperm morphology, especially examination of the acrosome and detection of nuclear vacuoles. In Australia (Beggs et al. 2013), trained sperm morphologists equipped with DIC microscopes now provide a commercial sperm morphology service for practicing veterinarians.

The interpretation of the findings of the assessment of sperm morphology is a two-step process. The percentage of normal and abnormal sperm is considered first. Across species, 30% total abnormal sperm is considered the threshold above which fertility is likely to be reduced. Thus in the absence of any physical abnormalities likely to affect reproductive function, males who produce ejaculates containing 70% or more normal sperm are considered likely to be fertile. However, the distribution and percentage of individual categories of sperm abnormalities should also be considered, especially in cases in which the percentage of morphologically normal sperm is less than 70%. Examining the frequency of specific sperm abnormalities (often referred to as the spermiogram) enables the veterinarian to assess the severity of dysfunction, and, in some cases, the likely cause of the problem (e.g., high proportions of retained proximal droplets in young males undergoing maturation of spermatogenesis postpuberty). Interpreting a spermiogram requires a good understanding of the stages and timing of spermatogenesis and epididymal transport and maturation. For example, spermatogenesis in the bull takes approximately 9 weeks followed by 2 weeks transport and maturation through the epididymis (i.e., it takes almost 2.5 months from differentiation of a spermatogonium to arrival in the tail of the epididymis ready to be ejaculated; see also Chapter 2). Thus, as this is a continuous process, the analysis of the distribution of abnormal sperm in a sample provides a 'snapshot' of the function

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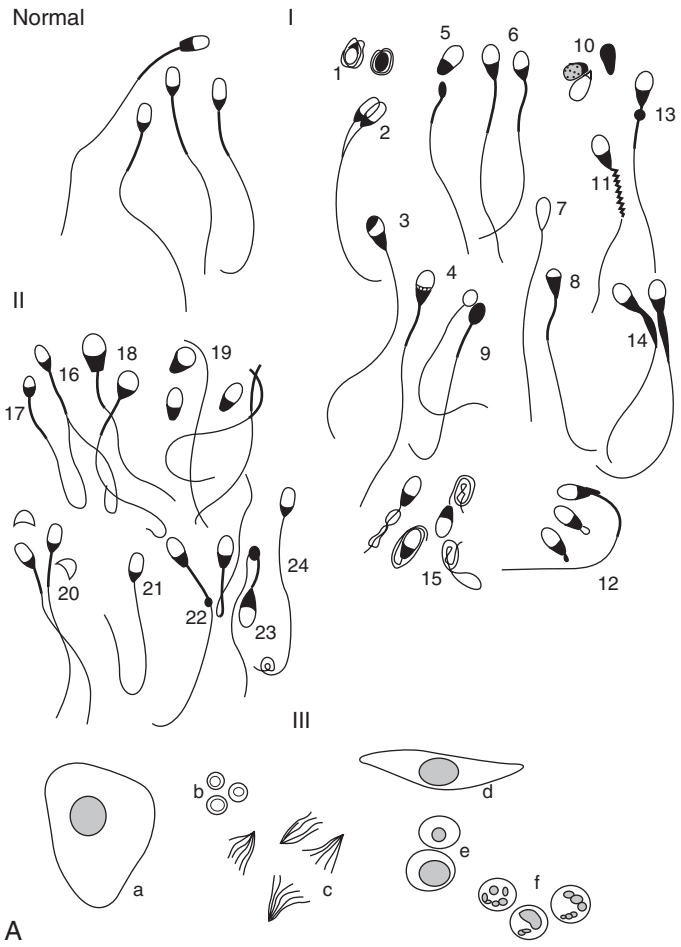

• **Fig. 35.12** (A) Classification of spermatozoal abnormalities into major and minor defects according to their effect upon fertility. (B) Field laboratory for bull semen examination. Note the small blow heater to the left on the bench to ensure that the entire area where semen samples are being handled, examined, and processed is kept warm. See Table 35.2 for key. (Adapted from Blom 1983.)

TABLE 35.2 Major and minor sperm abnormalities[a]

Major Abnormalities (Group I)			
1	Underdeveloped cells	9	Small abnormal heads
2	Double forms	10	Detached abnormal heads
3	Acrosome (knobbed acrosome) defects	11	Corkscrew midpiece defect
4		12	Other midpiece defects (incl. tail stump defect)
5	Diadem defects	13	
6	Decapitated sperm (active tails)	14	Proximal cytoplasmic droplet
7		15	Other thickened midpieces
8	Pear-shaped (pyriform) head	23	Coiled tails and Dag defect
	Head narrow at the base		Looped tail, enclosed droplet
	Head abnormal contour		Looped tail, enclosed droplet (= distal midpiece reflex) (not illustrated)

Minor Abnormalities (Group II)		Other Cells (Group III)	
16	Narrow heads	a	Epithelial cells
17	Small normal heads	b	Erythrocytes
18	Giant or broad heads	c	Medusa formations
19	Detached normal heads	d	Boat cells
20	Detached acrosomal membranes	e	Mononuclear cells
21		f	Neutrophils
22	Abaxial tails		
23	Distal cytoplasmic droplets		
24	Simple bent tail		
	Terminally looped tails		

[a]As defined by Blom (1983).

of the testes and epididymis over, in the case of the bull, the 2.5 months before sample collection. Saacke et al. (2000) demonstrated that after insulation of the testes of a bull for 48 hours (which mimics a range of common testicular insults), there was subsequently sequential appearance of specific sperm abnormalities, depending on the stage of development of sperm at the time of the insulation event. Semen collected within a week of the insult contained increased proportion of immotile and dead sperm, sperm with detached heads, and sperm with retained droplets (particularly proximal droplets). Samples collected between 1 and 3 weeks after the insult contained increased proportions of sperm with abnormal heads (pyriform) and nuclear vacuoles ('diadem' defect); and those collected after 3 weeks, in addition to these abnormalities, also contained sperm with abnormalities of the acrosome.

In some species, thresholds have been set for specific sperm abnormalities or categories of abnormality (e.g., bulls; Parkinson & Bruere 2007, Beggs et al. 2013). There is some evidence that morphologically normal sperm in ejaculates with a significantly increased frequency of abnormal sperm contain aberrations in their chromatin (Saacke 2008). Thus setting a threshold of 20% for major abnormalities seems reasonable. Categorising sperm abnormalities has been proposed according to:

- Site on the sperm (acrosome, head, midpiece, tail);
- Likely effect on fertility (major and minor; Blom 1983; Fig. 35.12; Table 35.2); or
- Whether abnormalities are compensable (sperm which are grossly abnormal and are unable to traverse the reproductive tract; increasing the total number of sperm in the inseminate can potentially compensate for the presence of these sperm), or uncompensable (sperm which are capable of binding to the ovum but, due to abnormalities in the sperm chromatin, cannot initiate fertilisation or normal embryo development), increasing the total number of sperm in the inseminate cannot compensate for the presence of these sperm (Saacke 2008) (Table 35.3).

Finally, in some cases, males will produce ejaculates containing a high proportion of a single sperm abnormality. These males are likely to have a heritable disturbance in spermatogenesis.

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TABLE 35.3 Compensable and uncompensable defects

Compensable Defects	Uncompensable Defects
Distal midpiece reflex	Proximal cytoplasmic droplets
Dag defect	Pyriform heads
Abnormalities of the mitochondrial sheath	Chromatin defects
Tail stump defect	Sperm head vacuoles
Tail defects	Macro- and microcephalic heads
Knobbed acrosome	Nuclear crest
Swollen acrosome	
Loose/detached heads	

Compiled from Evenson (1999), Saacke et al. (2000).

Assessment of Sperm Concentration

For field use, sperm density can most readily be estimated using a haemocytometer. Ram and bull semen should be diluted 1:100 in 0.9% saline/0.02% formalin solution. Other species whose semen is less dense may require lower dilution factors. The total sperm count is then derived as the product of volume and density. When a large number of semen samples require evaluation, such as occurs in AI studs, estimation of sperm density can be facilitated by the use of spectrophotometry, in which the optical density of the sample is compared with a calibration curve (Salisbury et al. 1943). Alternatively, electronic particle counters can be used, although the small size and flattened shape of the sperm heads make them relatively difficult cells to count.

In vitro Assessment of Semen Quality

An array of *in vitro* tests have been developed that enable investigation of the contribution of specific components of sperm function to the outcome of deposition of semen in the female reproduction tract (Rodriguez-Martinez 2007). These tests have been developed with the aim not only of improving, in particular, the sensitivity of identification of highly fertile males but also of subfertile or infertile males that appear to have normal sperm motility and morphology. The tests include:

- Computer-assisted analysis of sperm motility (CASA);
- *In vitro* fertilisation (IVF);
- Components of IVF such as assessment of sperm attachment to the zona pellucida;
- Assessment of sperm cell membrane integrity and mitochondrial integrity and functionality using various flurophores (e.g., the acylated membrane loading dye SYBR-14); and
- Sperm chromatin structure assays (SCSA), which enable the percentage of spermatozoa with denatured DNA to be determined.

However, the results of none of these *in vitro* tests have been shown to be highly correlated with fertility, and thus none are currently routinely used in breeding soundness examinations. Furthermore, although there have been major advances in our understanding of the role of seminal plasma proteins in sperm survival, fertilisation, and early embryo development (Rodríguez-Martínez 2011), the relationship between measures of the amount of specific seminal plasma proteins and fertility have not been sufficiently well established for incorporation into breeding soundness examinations.

Assessment of Libido and Serving Ability

This component of the breeding soundness examination focusses on assessment of the male's ability to repeatedly mount and serve normally and almost always requires direct observation of the behaviour of the male when exposed to a free-standing female in oestrus or an appropriately restrained female. Unfortunately, it is not always assessed because of the practical difficulties of conducting the assessment, sourcing and restraining mount females, and the time (at least 10–40 minutes) required to observe each male express their mating behaviour. However, when investigating a suspected subfertile or infertile male or when a client wants to mate a few selected males to a large group of females for a short period, especially if the females have been treated to synchronise ovulation, assessment of libido and serving ability is strongly recommended. Further, it must be appreciated that the definitive test of whether a male has a normal musculoskeletal system is observation of him attempting to mount and serve. The author has been chased out of a yard by a bull that subsequently in a serving ability test showed evidence of marked hindlimb paresis caused by severe osteoarthritis affecting the lumbar spine.

Assessment of mating behaviour should be conducted in a small area (enclosed pen, holding yard) with good footing, and, wherever possible, the test site should be familiar to the male(s). Veterinarians must position themselves to ensure that they are able to clearly observe whether intromission and ejaculation occurs normally, especially in species (rams, bulls) in which the duration of coitus is very short. Testing young males that have not been mated before can be problematic as sexual behaviour is learned. Providing virgin males with a short period of sexual experience before testing therefore generally increases the expression of sexual behaviour. Whenever possible, males should be given the opportunity to serve more than once, especially older males, because if, for example, they have degenerative joint disease affecting the lower back and/or hindlimbs, they may serve normally once but, due to pain/loss of motor function, they will not serve again.

Although the veterinarian can be confident of the serving ability of males who are observed to normally serve the mount female several times, considerable caution must be applied in the interpretation of males that fail to express sexual behaviour or express only limited behaviour. Males can be readily inhibited by a range of stressors (e.g., being recently transported to the site where testing is to be conducted or being mixed with other males, especially older males). Further, for example, in cattle, *B. taurus* bulls typically achieve a significantly higher number of services in a yard test than *B. indicus* bulls.

Routine assessment of libido and serving ability is most commonly conducted on young *B. taurus* bulls before selection as sires. Also, it is routinely conducted on older herd sires, especially in intensively managed beef herds, to identify bulls with premature spiral deviation of the penis and degenerative joint disease affecting the lower back or hindlimbs. Practical guidelines for this procedure, including design of the modified head bail to restrain the mount females, have been developed (Beggs et al. 2013). Mount females should be selected on the basis that they are not pregnant, at least two-thirds the weight of the bulls to be tested, of quiet temperament, and have been vaccinated for venereal diseases. The number of mount females required is about half the number of bulls to be tested, and during the testing the welfare of the mount females

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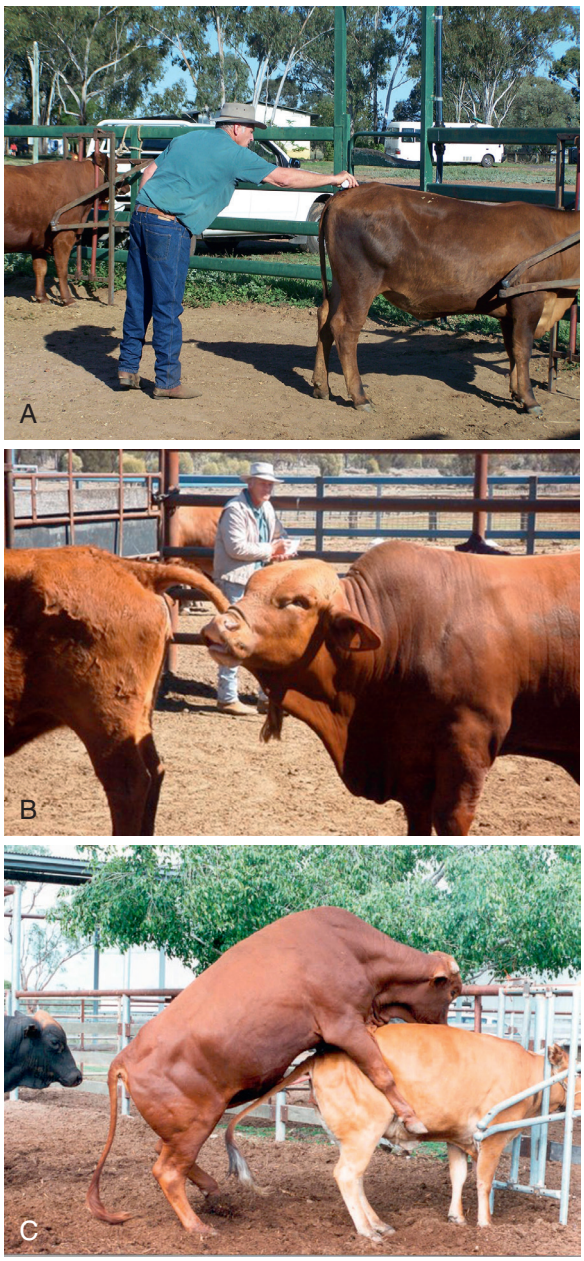

• **Fig. 35.13** Service testing of bulls. (A) Appropriate size mount animals of good temperament are restrained in purpose-built bails and used for a defined numbers of mounts. (B) Bulls are observed during their approach to the mount cow and (C) during mounting to ensure that normal intromission and ejaculation occur. (Photos courtesy Dr John Bertram.)

must be regularly monitored. After infusion of obstetrical lubricant into the vagina the haltered mount females are drawn into and restrained in purpose-built bails (Fig. 35.13). Barth et al. (2004) have developed an innovative intravaginal AV that allows both assessment of libido and serving ability and enables collection of a sample of semen for microscopical examination. Blockey (1978) demonstrated that the number of services achieved by *B. taurus* bulls in a 40-minute yard test was significantly correlated with the pregnancy rate achieved after natural mating. However, the ability to reliably predict the pregnancy rate that will be achieved based on the performance of a bull in a serving test is limited because of the many other factors that contribute to this outcome. Assessment of libido and serving ability is also commonly conducted on rams, especially in intensively managed flocks employing low male to female ration and short mating periods. The procedure used is similar to that described for bulls.

Overall Determination of the Likely Fertility of a Breeding Male

Having completed a breeding soundness examination a veterinarian must then determine by careful consideration of all findings whether the male:

- Is likely to be fertile;
- Should only be used as a terminal sire;
- Should be reexamined (typically 6–9 weeks later) to better define its fertility status; or
- Is likely to be subfertile or infertile.

This is a professional opinion based on the findings of the examination performed (see Fig. 35.1). The interpretation of measurements such as scrotal circumference or diameter, percentage of progressively motile sperm, percentage of morphologically normal sperm, and number of services achieved in a serving ability test may, in some species, be supported by threshold values derived from research that has defined the relationship between these measures and mating outcomes (Coulter & Kozub 1989, Holroyd et al. 2002). For those males that are diagnosed as being subfertile or infertile/sterile, the veterinarian must provide the client with recommendations for their future management which could include reexamination, therapeutic management, temporary or permanent withdrawal from service, or culling. Various methods of classification of breeding males have been developed, particularly for bulls (Hopkins & Spitzer 1997, Parkinson & Bruere 2007, Beggs et al. 2013), to enable standardised reporting of the findings of breeding soundness examinations. Whatever system is used to report the findings of a breeding soundness examination, it is important that the wording used is unambiguous, and clear definitions are provided for the terms used.

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36

Abnormalities Affecting Reproductive Function of Male Animals

TIMOTHY J. PARKINSON AND MICHAEL MCGOWAN

Reproductive abnormalities causing subfertility and infertility in male animals have classically been divided into two main classes: conditions causing partial or complete failure of normal service (*impotentia coeundi*) and conditions causing partial or complete inability to impregnate normal cycling females (*impotentia generandi*). The first group can be further divided into conditions causing unwillingness or inability to mount and conditions that prevent normal copulation from occurring despite normal libido. Superimposed upon both groups are considerations of whether the subfertility/infertility is due to a primary pathological abnormality or suboptimal husbandry or breeding management. Fig. 36.1 outlines the diagnostic pathways for the major causes of subfertility/infertility in bulls and can be readily adapted to other species.

Abnormalities of Coitus

Disorders associated with partial or complete failure of coitus can be considered under the following:

- Immaturity or inexperience of breeding male;
- Inability or unwillingness of the breeding male to mount;
- Inability of the breeding male to achieve intromission; and
- Failure of the breeding male to ejaculate.

Immaturity and Inexperience

Young breeding males may be presented for failure to mate simply because they are peripubertal and/or sexually inexperienced. When immaturity is suspected as the cause of poor libido, little can be achieved other than to ensure good husbandry management and exposure to cycling females. Clients must be counselled about the important role of genetics in onset of puberty: males who express delayed onset of puberty are likely to sire offspring with this trait.

Hormone therapy has been suggested. Administering large doses of human chorionic gonadotrophin (hCG) (5000–10,000 IU) or gonadotrophin-releasing hormone (GnRH) to stimulate increased secretion of testosterone has been suggested. However, considerable caution should be exercised in the use of such treatments as they may induce aggressive behaviours. Moreover, hCG, although having luteinising hormone (LH)-like properties, is not the same substance as LH and may impair spermatogenesis due to treatment associated testicular oedema. In general, the efficacy of such treatments is low. Most animals fail to respond at all, a few exhibit a short period of enhanced libido, but only in very few cases can success be attributed to the treatment.

Lack of sexual experience and/or suboptimal rearing conditions can adversely affect the expression of sexual behaviour in young males. For example, when bull calves are reared in groups, they regularly exhibit mounting behaviour as puberty approaches and usually learn to copulate quite quickly. However, when reared in isolation, such mounting behaviour does not occur and can take some time to be learnt, especially in those AI stations where steers are used as mount animals. Similarly, young colts in racing yards may be violently dissuaded from exhibiting male behaviour by their grooms. As a consequence, such negative behavioural reinforcements have to be reversed before these young horses can be used for mating.

Unwillingness to copulate can also result from poor mating management. Slippery floors, roofs that are too low, females that are too big, and stockmen that are insensitive in their handling of their charges can all contribute to unwillingness to copulate. Similar problems pertain among companion animals. Tomcats frequently become conditioned to mating in one particular environment and, if that environment should later be changed, then they may become unwilling to mate. Dogs, who are frequently travelled before mating, can also have stress-induced impairment of libido. Finally, young males of many species, notably the boar and bull, are frequently unwilling to mate if within the sight or sound of an older, more dominant, male.

Inability or Unwillingness to Mount

Most lesions affecting locomotion impair ability and willingness to copulate. Lesions of the back and hindlimbs are the most important of such incapacities, but, for example, in the boar, in which the forelegs are used to clasp the female, painful lesions of the carpus can also impair mating.

In production animals and horses, foot lesions are probably of greatest significance. Gross pathology of the foot, such as sole abscess, laminitis, white line disease, infectious interdigital dermatitis, and interdigital fibroma, cause significant pain, and, as a consequence, the affected male will either be unwilling to mate or its frequency of mating will be markedly reduced. Poor conformation of the foot is less obvious but equally important as a cause of low libido. Animals with overgrown hooves, in which the distribution of weight has been adversely affected, are frequently unwilling to mount or, if they do mount, are unwilling to remain mounted for long enough for successful copulation to occur. For this reason, valuable sires in AI centres receive considerable attention to the conformation

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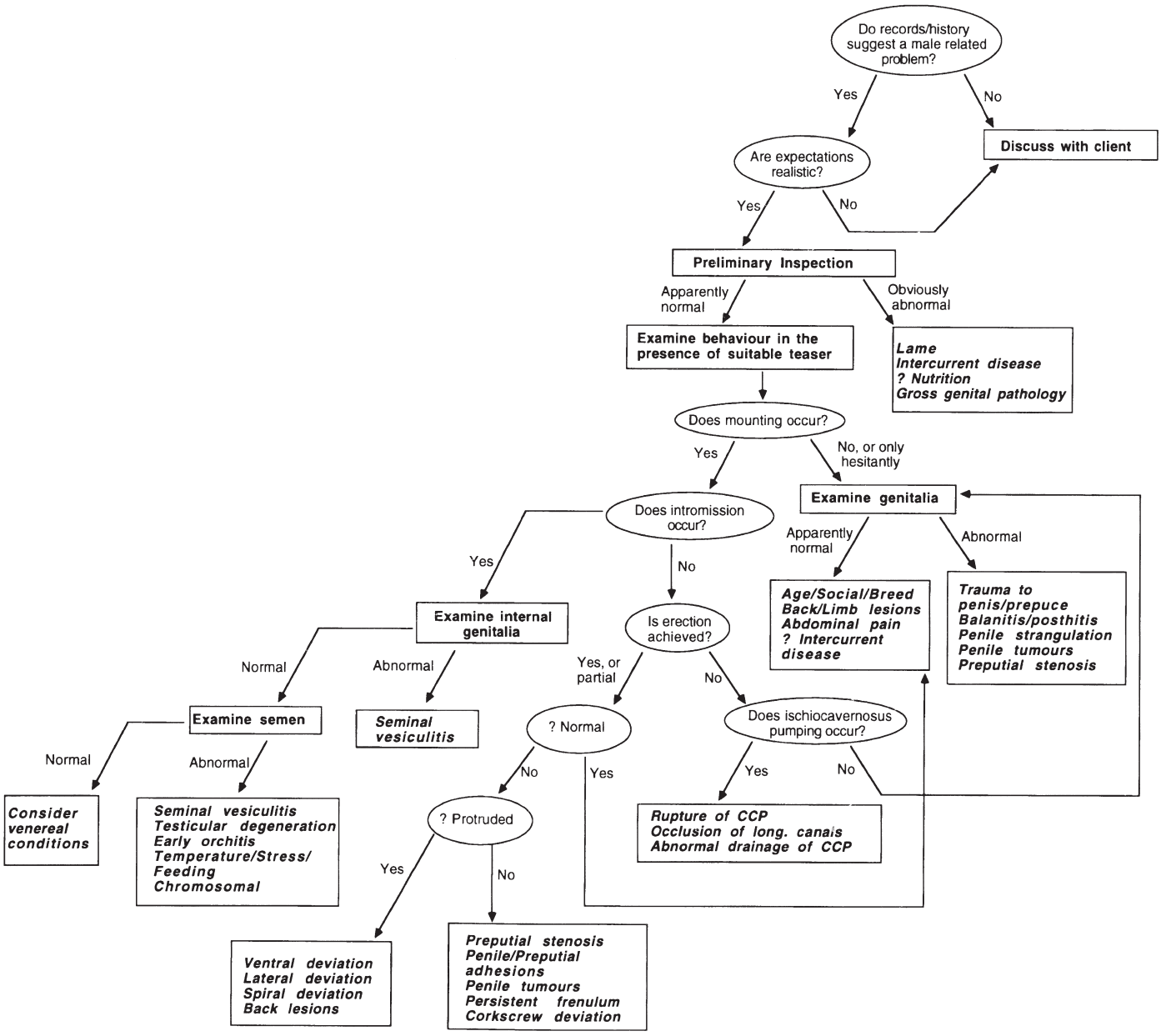
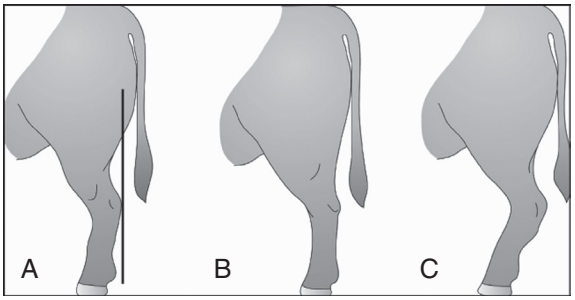


• **Fig. 36.1** Outline of the diagnostic pathways for the major causes of subfertility/infertility in bulls. CCP, corpus cavernosum penis. (With permission from Parkinson 1991.)

of their feet. By contrast, many farm sires, especially bulls, receive little such attention until overt lameness has developed.

Lesions of the joints of the hindlimb are important locomotor causes of impaired mounting behaviour in all species. Abnormalities of hindlimb conformation are also important. Older bulls whose hocks are excessively straight (a relatively common problem: Fig. 36.2a) or oversickled (Fig. 36.2c) commonly have degenerative lesions of the hock and are consequentially unwilling to mount.

Similarly, any lesion of the vertebral column affects ability to mate; the flexure of the spine that occurs in the bull during the ejaculatory thrust is shown in Fig. 36.3. In young bulls (first mating season) that are overzealous in their early attempts to mate, the lumbodorsal fascia may rupture. In this condition the pain caused by the rupture of the fascia is such that the bull will refuse to



• **Fig. 36.2** Conformational abnormalities of the hocks (A) too straight or (C) too sickled in comparison with normal (B) predispose to reduced mating ability in older bulls. (With permission from Parkinson & Bruere 2007.)

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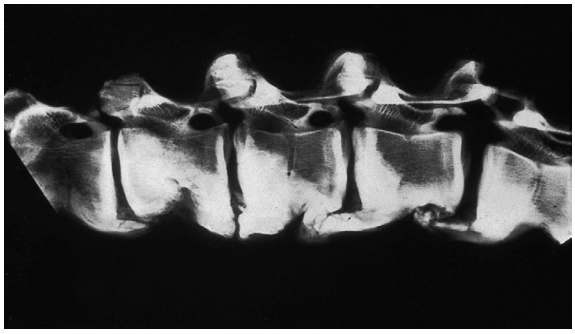
• **Fig. 36.3** Flexure of the spine of the bull during the ejaculatory thrust.



• **Fig. 36.4** Radiograph of the lumbar spine of an aged bull. Several of the vertebrae display significant evidence of osteoarthritis.

attempt to mount. It can be diagnosed by palpation of crepitus in the lumbodorsal region, presence of swollen firm lumbar muscles, or wasting of these muscles.

As males age, injury and degeneration of the vertebral column becomes an increasingly common cause of impaired mounting, and thus it is strongly recommended that an assessment of mating ability be routinely included in the breeding soundness examination of aged males. Breeding males with back pain may mount, but frequently dismount again quite quickly. They may rest their chin on the sacrum of the mount animal or female and tend to lever themselves upwards during mounting; they often fail to clasp the female firmly with the forelegs and their dismount is clumsy. Affected bulls are unwilling to make the ejaculatory thrust and may mount without ejaculating, or they may mount/ejaculate once but are disinclined to serve again. These bulls exhibit similar behaviour if AV collection of semen is attempted (Almquist & Thomson 1977).

A major cause of back pain in bulls is progressive degenerative joint disease affecting the intervertebral joints (Fig. 36.4), causing several related syndromes of incapacity. These conditions, which are common in housed bulls receiving diets that are relatively high in calcium (Krook et al. 1969), rarely present at less than 7 years of age (Bane & Hansen 1962). First, progressive growth of exostoses can merely make the bull appear 'stiff', and they may fail to mount and clasp the hindquarters of the cow 'cleanly'. More seriously, spondyles of bone can fracture, usually during mounting, causing immediate, acute, back pain that is accompanied by a complete, but usually temporary, unwillingness to mount. Very dramatically, when complete bridges of bone form between several adjacent vertebrae, flexing forces upon the spine can cause fractures within the spinal column, typically straight across a vertebral body. Such fractures typically occur at the moment of ejaculation, whereupon the bull becomes immediately paraplegic by spinal cord severance. The bull therefore collapses off the cow or mount animal into a dog-sitting position and exhibits partial or complete loss of sensation of its hindlimbs, depending on the severity of the spinal cord lesion.

Abnormalities of gait are a further cause of inability to mate. Details of such are beyond the scope of this chapter (for a review, see Watson 2007) other than to highlight three heritable conditions of the bull: spastic paresis, spastic syndrome, and congenital ataxia in Charolais cattle. Similar conditions occur in many other domestic species.

Finally, it should be noted that many conditions that would normally be expected to present with signs other than unwillingness to mount can, if neglected, result in a sexually uninterested animal. Lesions of the penis that prevent normal coitus (see later in this chapter) are of particular significance in this regard.

Inability to Achieve Intromission

Inability to achieve intromission is a relatively frequent cause of infertility in domestic animals. Conditions that cause failure of copulation include failure to achieve an erection, abnormalities of erection that prevent intromission, and lesions of the penis and prepuce that prevent protrusion of the penis. Most of the conditions can be differentiated relatively easily, and a prognosis can usually be given at an early stage of investigation.

Failure of Erection

Erection is achieved by contractions of the ischiocavernosus muscles pumping blood into the corpus cavernosum penis (CCP). Because the CCP is, essentially, a blind-ending chamber, whose venous drainage is close to its arterial supply in the crura of the penis, contraction of the ischiocavernosus muscles results in occlusion of the venous return, and blood is forced into the arteries, thereby raising the hydrostatic pressure within the CCP. The hydrostatic pressure thus generated produces the lengthening and stiffening of the penis that characterises erection (Beckett et al. 1975). Thus, if any aspect of the vascular system of the CCP is perturbed, failure of erection ensues. Two main classes of abnormalities occur: those that allow blood to leak from the CCP and those that prevent normal access of blood to the CCP.

Ruptured Penis (Rupture of the CCP, Fractured Penis, and Broken Penis)

This condition has been comprehensively reviewed by Ashdown (2006). It is a common abnormality of the genital system of the bull (Hughes & Oswald 2007), and it also occurs sporadically in boars and rams. Rupture of the tunica albuginea occurs spontaneously if pressures within the CCP rise substantially higher than the pressures achieved during normal copulation (Noordsy et al. 1970, Beckett et al. 1974). Such abnormal increases in pressure can occur if the penis is suddenly subjected to shearing forces – for example, by the cow moving suddenly at the moment of ejaculation or by the ejaculatory thrust being misdirected against the perineum of the cow rather than into the vagina. Rupture (Fig. 36.5A and B) occurs most commonly either in the region of the insertion of the retractor penis muscle or on the dorsal aspect of the distal sigmoid flexure, where the trabeculae of the CCP are relatively weak. In rams, it is more common to see rupture of the CCP near the root of the penis, above the proximal sigmoid flexure, presumably as a result of being butted from behind during mating.

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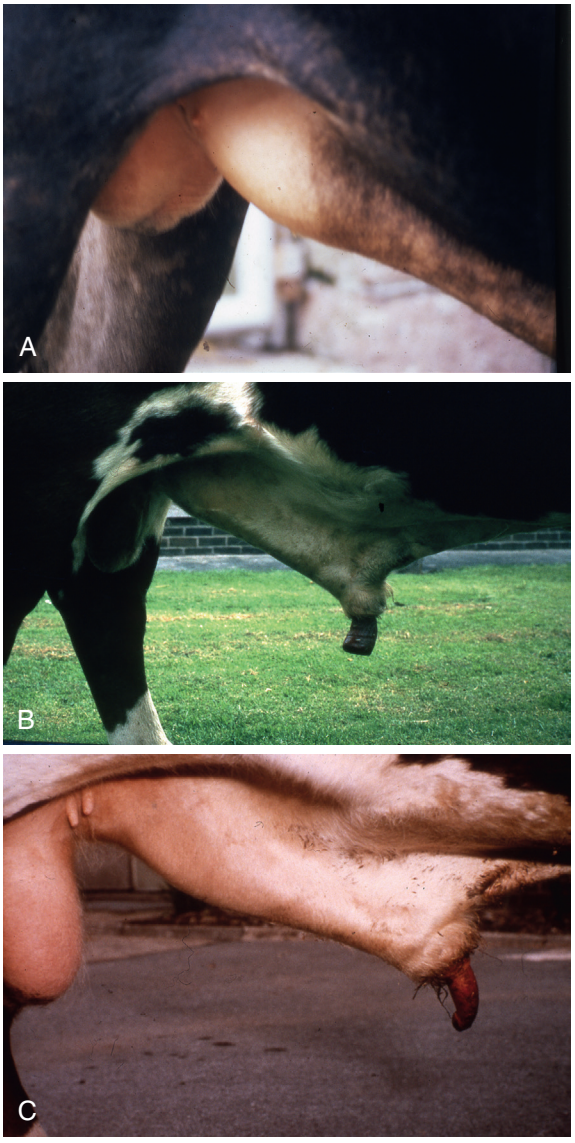
• **Fig. 36.5** Ruptured corpus cavernosum penis. (A) Site of rupture of the tunica albuginea. (With permission from Parkinson & Bruere 2007.) (B) Peripenile haematoma resulting from the rupture of the CCP, sited close to the insertion of the retractor penis muscle.



• **Fig. 36.6** (A) Prescrotal swelling due to haematoma formation after rupture of the CCP in a bull. (B) Prolapse of the preputial mucosa after rupture of the penis in a Hereford bull. (C) Prolapse of the penis secondary to rupture of the penis.

Rupture of the penis occurs more commonly in younger bulls, possibly due to a combination of over-vigorous penile thrusting and mating inexperience. Immediately after rupture, the animal may be noticeably subdued and may display signs of mild discomfort, including shortness of gait. Many, but by no means all, affected animals are subsequently unwilling to mount. Haemorrhage occurs from the site of rupture, with haematomata formation in the surrounding tissues, cranial to the scrotum in ruptures of the distal sigmoid flexure (Fig. 36.6A) and behind the scrotum with proximal ruptures. These haematomata can become very large, especially if mating behaviour is not immediately inhibited. Distal ruptures are also characterised by preputial oedema, which is often sufficiently severe to cause prolapse of the prepuce (see Fig. 36.6B). Occasionally, the penis itself may be prolapsed (see Fig. 36.6C). In light-coloured bulls, subcutaneous haemorrhage may be visible over the site of rupture of the CCP. Urination is not affected.

The haematoma is initially soft and fluctuant, but later, as the clot becomes organised, it becomes very firm. It is not possible to determine the extent of the haematoma during the initial phase when the prepuce is oedematous, so assessment must take place after the oedema has subsided. If untreated, a substantial proportion of haematomata can become infected, with subsequent formation of an abscess (Fig. 36.7A), whereas others develop fibrous adhesions between the penis and prepuce and within the fascial planes of the prepuce (see Fig. 36.7B). Diagnosis is relatively straightforward in recent cases, but chronic cases must be differentiated from preputial trauma, abscessation within the sheath, and 'water belly' after rupture of the distal urethra. Peripenile haematomata may also be caused by blunt force trauma to the sheath area during fighting (Noordsy et al. 1970).

Due to the time taken for recovery and common sequelae (adhesions, abscess, erectile dysfunction), most affected bulls, except for valuable animals, should be culled. When treatment is considered, several alternatives need to be considered, whose relative merits and demerits are still the subject of debate. Conservative treatment, consisting of sexual rest for 90 days, immediate antibiotic therapy to prevent the haematoma developing into an abscess, non-steroidal antiinflammatory treatments, and daily cold hosing of the sheath area, has been reported in some surveys to allow as many as 50% of bulls to regain the ability to serve normally. However, other surveys have indicated that a successful response to conservative therapy occurs in as few as 10% of animals. Selection of cases to which conservative treatment is applied may account for some of the variation between surveys, for bulls with relatively small, freely

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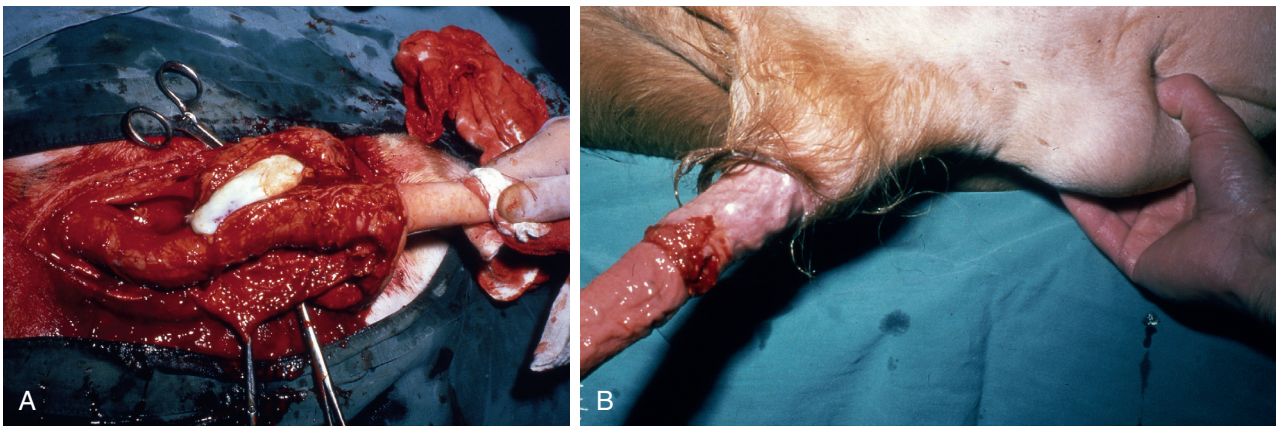
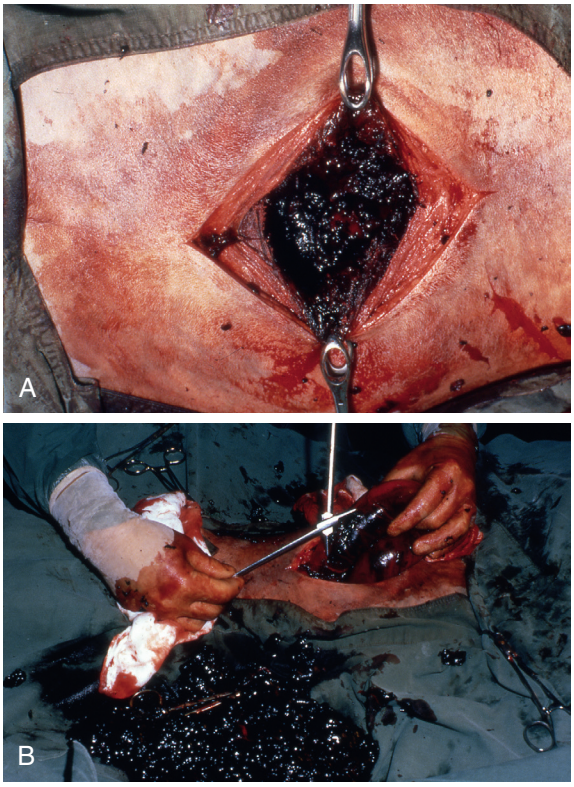


• **Fig. 36.7** (A) Secondary abscess formation in a peripenile haematoma in a bull. (B) Preputial fibrosis in a yearling bull with a small penile haematoma. The fibrotic lesion prevented protrusion and required resection.

movable, circumscribed haematomata that are little bigger than the diameter of the normal penis respond best to conservative treatment. Larger haematomata carry a greater risk of abscess formation and development of peripenile adhesions. Surgical treatment of such cases is best confined to evacuation of the haematoma, with strict attention to asepsis (Fig. 36.8A and B). More radical surgery, in which the site of rupture of the tunica albuginea is isolated, resected, and sutured, has been suggested (Morgan 2007, Wolfe 2015a). In either case, surgery should not be delayed for more than 7 days after injury, as adhesions can become extensive thereafter. Sexual rest after surgery should only last for a few days, for a long period of inactivity also promotes peripenile adhesions. Affected bulls should be managed in a secure pen and regularly teased to induce penile movement that will reduce the risk of peripenile adhesions.

Peripenile adhesions are likely to be more severe if the clot is not removed, and even quite small areas of fibrosis can prevent penile protrusion. A small proportion of affected bulls develop ectopic veins that drain the CCP through the site of rupture, thereby preventing vascular engorgement of the CCP (Young et al. 1977). In such cases, surgical location of the site of the venous drainage and closure of the vein is often feasible (Wolfe 2015a) for, unlike congenital venous drainage of the CCP, such acquired cases usually only possess a single abnormal vein. Damage to the dorsal nerve of the penis, either at the time of injury or during surgical repair (Morgan 2007) is a well-recognised sequelae of rupture of the penis. Affected bulls often fail to achieve intromission, and, if they do, they will fail to ejaculate.



• **Fig. 36.8** (A, B) Surgical evacuation of a peripenile haematoma in a bull.

Abnormal Venous Drainage of the CCP

This rare condition (Young et al. 1977, Ashdown et al. 1979a) of young bulls presents as an animal with normal libido that is eager to mount but never achieves erection or intromission. Observation of the mating behaviour of such bulls reveals excessive ischiocavernosus muscle contractions before and during mounting, to such an extent that the tailhead appears to be pumping up and down, although the penis remains flaccid. The cause of this condition is almost invariably congenital failure of occlusion of the veins that drain the CCP (Ardalani & Ashdown 1988). A similar condition occurs in boars, in which the CCP drains into the corpus spongiosum glandis (Ashdown et al. 1982).

Diagnosis of the condition can generally be made on clinical signs and history alone. However, if further confirmation of the cause of the condition is required, the presence of ectopic veins can be confirmed by injecting radiographic contrast media into the CCP of the anaesthetised bull and then observing drainage of contrast into the dorsal veins of the penis (Fig. 36.9A and B). Because there are nearly always many small veins along both lateral edges of the CCP, surgical correction is rarely possible.

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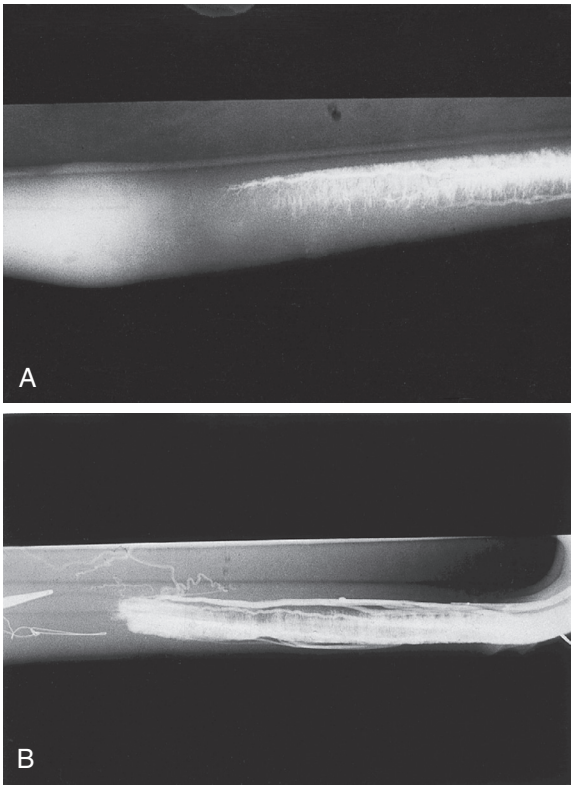
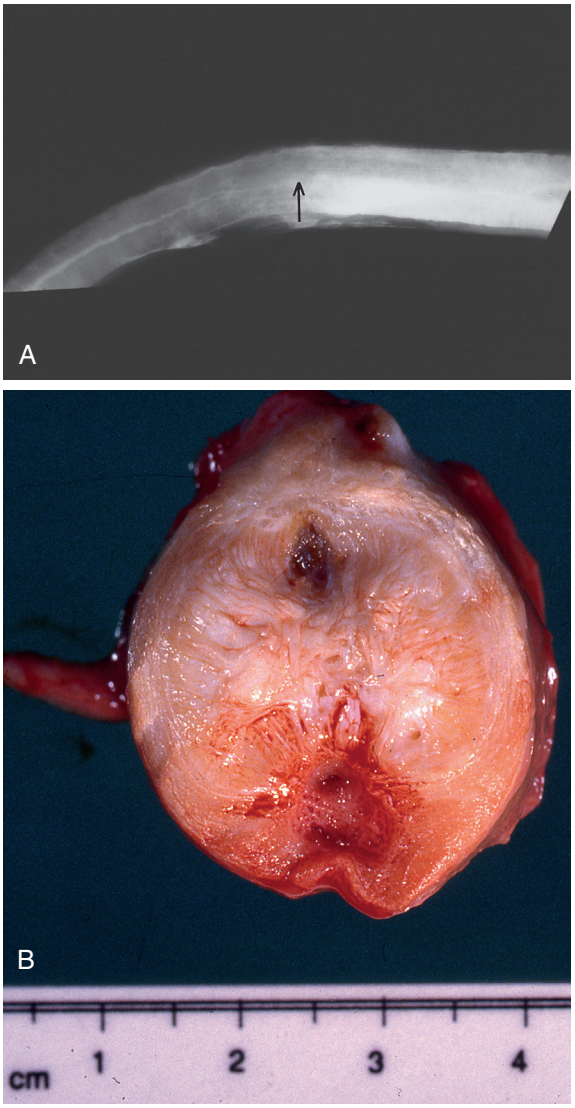


• **Fig. 36.9** Abnormal venous drainage of the CCP. Radiographs of the penis of (A) a normal and (B) an impotent bull after the injection of contrast medium into the CCP near the root of the penis. In the normal animal the contrast medium is contained within the CCP, but in the impotent animal, contrast medium is leaking into the dorsal vein of the penis, indicating the presence of ectopic veins draining the CCP.



• **Fig. 36.10** Blockage of the longitudinal canals of the penis of a bull. (A) Contrast radiography demonstrates the point at which the lumen of the dorsal longitudinal canal is blocked. (B) Transverse section of the penis, showing occlusion of the dorsal longitudinal canal with atheroma-like material.

Occlusion of the Longitudinal Canals of the Penis

During erection of the bovine penis, increased blood pressure produced by contractions of the ischiocavernosus muscles is transmitted throughout the length of the CCP by the longitudinal canals. Congenital absence or acquired blockage of these canals therefore prevents erection (Ashdown et al. 1979b). Both young bulls and animals that have been successfully used as sires over a number of years can be afflicted with this condition. Young bulls present in a very similar way to animals with abnormal venous drainage of the CCP, i.e., the penis is flaccid during attempted mating. In young bulls the condition is typically caused by a congenital failure of cannulation of the short segment of the single, dorsal longitudinal canal (between the point of fusion of the two crural canals and the origin of the first of the lateral branches that form the ventral canal). In such bulls, the condition is diagnosed by observation of mating behaviour, when the penis remains flaccid despite considerable ischiocavernosus activity. It can be differentiated from abnormal drainage of the CCP by palpation of the base of the penis. Although the great majority of the penis is flaccid, a short length of turgid tissue is present in the part of the penis proximal to the occlusion (Fig. 36.10A). This can be appreciated by palpating the root of the penis just beneath the tailhead, where the penis may become so turgid that the animal may resent it being touched. There is no treatment for this condition and affected bulls should be culled.

The condition in older bulls typically presents as a bull with normal libido that is observed to have a flaccid penis during attempted mating. Affected bulls typically have a history of normal service behaviour. The cause of the condition is blockage of the longitudinal canals by fibrinous or, more usually, atheromatous material (see Fig. 36.10B). This condition also occurs in rams, in which the CCP can rupture proximal to the site of obstruction, causing a peripenile haematoma in the region of the escutcheon. Similar ruptures occur infrequently in affected bulls.

Deviations of the Penis

Premature Spiral Deviation of the Penis (PSPD; Corkscrew Penis)

This is one of the most common disorders of the bovine penis (Hughes & Oswald 2007). In this condition, spiralling of the tip of the penis (which normally does not occur until the time of ejaculation) occurs before intromission (Fig. 36.11A), usually when the penis first touches the perineum of the cow (Ashdown & Coombs 1967, 1968, Ashdown et al. 1968; Seidel & Foote

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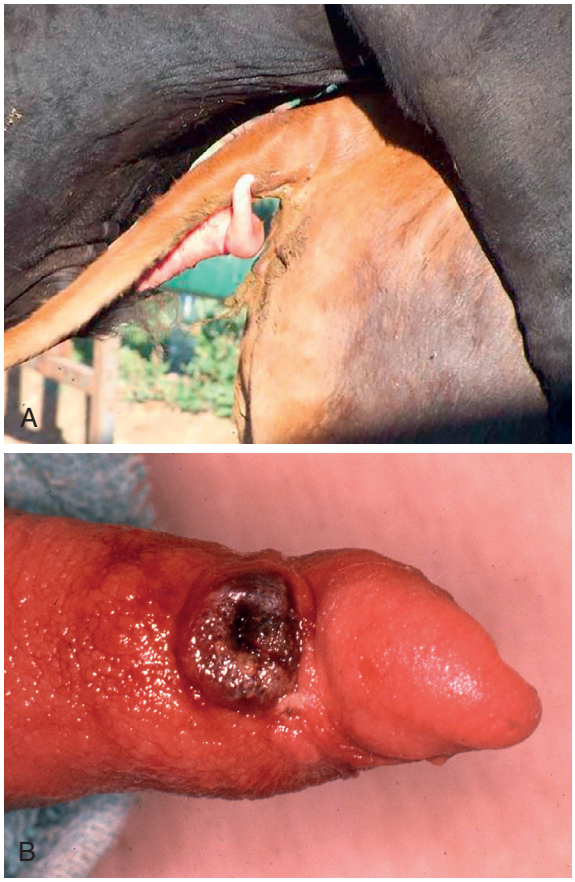

• **Fig. 36.11** (A) Premature spiral deviation of the penis (PSPD) manifested after mounting but before intromission. (B) Traumatic ulceration of the glans penis secondary to spiral deviation of the penis.

1967, 1969) or, occasionally, when the penis is still within the prepuce. Premature spiralling of the penis prevents intromission.

The onset of the condition may be at any age, although it is uncommon in yearlings and is most common in animals when they are 3 or 4 years old (Pearson & Ashdown 1976, Hughes & Oswald 2007). Hence, it is common for affected bulls to achieve normal fertility in their first year of use, then to start exhibiting premature spiralling of the penis during their second or third year. The condition is progressive, in as much as affected animals become less and less likely to achieve a normal erection with advancing age. The condition occurs more commonly in polled than horned bulls and is generally regarded as being inherited (Blockey & Taylor 1984). However, the mode of inheritance has not been proven, so culling the ancestors of affected bulls may not be warranted (Steffen 1997).

Premature spiralling of the penis can only be diagnosed by conducting a serving ability test and observing the bull attempting to serve multiple times. It is not uncommon for affected bulls to mount normally on their first attempt. The author (MMcG) has observed an affected bull show premature spiral deviation of the penis on 11 consecutive serves and then achieve normal intromission with ejaculation on the 12th serve. Affected bulls should be classified as being subfertile, as use of these bulls will cause an increase in the proportion of cows diagnosed nonpregnant or likely to calve late in herds with defined mating periods.

Frequent premature spiralling can result in the penile integument becoming traumatised as it comes into contact with the perineum of the cow. The pain caused by the consequent ulcer on the glans penis (see Fig. 36.11B) may, in time, impair the libido of the bull to the extent that he will not mount. The cause of premature spiralling of the penis has not been conclusively determined; it has variously been considered to be due to an anatomical deficiency of the dorsal apical ligament of the penis (Walker 1970), premature attainment of maximal blood pressure in the CCP, or even a neurological lesion. Given that this is a condition that is expressed in older bulls without any history of injury to the penis, it is feasible that the cause is congenital abnormal development of the dorsal apical ligament that predisposes to progressive weakening of the ligament with successive breeding seasons. Correction of the condition has been described by suturing the dorsal apical ligament to the tunica albuginea with alternating catgut and stainless steel sutures (Ashdown & Pearson 1973a) or by placement of a fascia lata graft into the ligament (Gilbert et al. 2004). However, because of the evidence that this condition is heritable, surgical repair is not recommended.

Lateral Deviation of the Penis

Lateral deviation of the penis is often attributed to injuries to the tunica albuginea of the penis but may arise from inadequate development of the dorsal apical ligament of the penis or congenital defects of the tunica albuginea; the condition has been reproduced experimentally by removal of the apical ligament of the penis in adolescent bull calves (Eurides et al. 2015). Hence, it has been suggested that some cases of right lateral deviation may sometimes be an early manifestation of premature spiral deviation of the penis. Treatment is occasionally successful, but is generally unrewarding (Boyd & Henskelka 1972, Walker & Vaughan 1980) because of the difficulty in identifying the site of the causal lesion. For valuable sires, collection of semen by electroejaculation could be considered, but the veterinarian must be confident that the problem is likely to have resulted from injury and does not have a heritable basis.

Ventral Deviation of the Penis

This condition, which is sometimes referred to as the 'rainbow' deviation (Fig. 36.12A), is commonly caused by persistence of the penile frenulum. In the absence of a persistent frenulum, the condition usually arises from defects in the fibrous architecture of the tunica albuginea. Such defects can be congenital but can also arise from injuries to the penis that result in scar formation within the tunica (see Fig. 36.12B). True ventral deviation of the penis must also be differentiated from partial failure of erection, when the penis appears deviated ventrally because of its flaccidity.

Congenital Abnormalities of the Penis Preventing Protrusion

Congenital shortness of the penis (Fig. 36.13) occurs rarely and results in failure to achieve intromission (Roberts 1986). Similarly, congenital shortening of the retractor penis muscles can cause inability to protrude the penis. Although treatable by myectomy, such abnormalities are probably inherited (DeGroot & Numans 1946), so surgery should not be undertaken in future breeding animals.

Persistence of the Penile Frenulum

Persistence of the penile frenulum is most frequently encountered in bulls (Ashdown 1962, Carrol et al. 1964; Fig. 36.14A and B), but has been reported in dogs (Joshua 1962, Johnston

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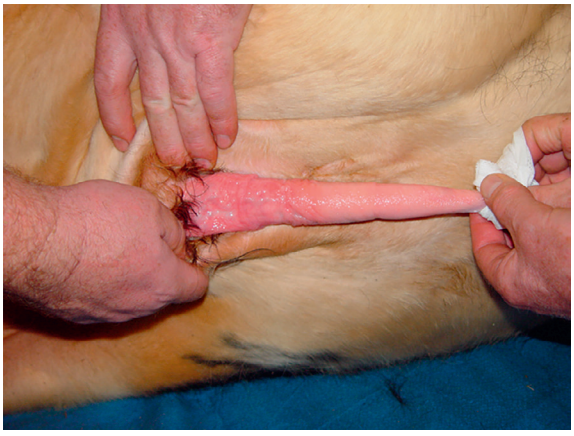
• **Fig. 36.12** (A) Ventral (rainbow) deviation of the penis. (B) This can sometimes be caused by localized fibrosis. This animal was treated by excision of the lesion. (With permission from Parkinson & Bruere 2007.)



• **Fig. 36.13** Congenital shortness of the penis, which prevents intromission, is a rare abnormality of the bull.

et al. 2001) and boars. In bulls the frenulum is present at birth and should begin to break down by 4 weeks of age. Separation should be complete by 8 to 11 months of age. Persistence beyond 11 months is considered pathological (Steffen 1997). Persistent frenulum results in deviation of the glans penis, preventing full protrusion of the penis or a marked ventral deviation of the penis. Transecting the frenulum after ligating the frenular blood vessels if they are sufficiently prominent gives a good prognosis for the recovery of breeding ability (Elmore 1981, Cox 1982). There is evidence that the condition is heritable, possibly as an autosomal recessive gene in cattle (Elmore et al. 1978, Steffen 1997), so the bull's sons should not be retained for breeding.

• **Fig. 36.14** Persistent frenulum in the bull. (A) Single frenulum resulting in deviation of the penis. (B) Double frenulum in an 18-month-old Friesian bull.

Juvenile Fusion of the Penis and Prepuce

At birth, the integument of the penis and the penile part of the prepuce are fused but, during the prepubertal period, the bridging connective tissue normally breaks down (Ashdown 1962). When the connective tissue remains substantially intact, the penis cannot properly be protruded, such that only a few centimetres appear through the preputial orifice. This condition is most frequently encountered in bulls that are reared in isolation (Cox 1982). The condition is frequently self-limiting once sexual activity begins, although it may result in persistence of the frenulum. It must be differentiated from other causes of adhesions between the penis and prepuce.

Hypospadias

Hypospadias has been reported in the dog (Ader & Hobson 1978, Hayes & Wilson 1986), cat (King & Johnson 2000), boar (Langpap 1962), bull (Saunders & Ladds 1978, McEntee 1990), goat (Sahay et al. 1986, Rajankutty et al. 1994), and ram (Dennis 1979, Smith et al. 2006). This congenital abnormality of the penis results from the partial or complete failure of the two urethral folds to fuse to form the extrapelvic urethra, and failure of the median raphe of the perineum, scrotum, penis and prepuce to develop (Baskin 2000, Yamada et al. 2003).

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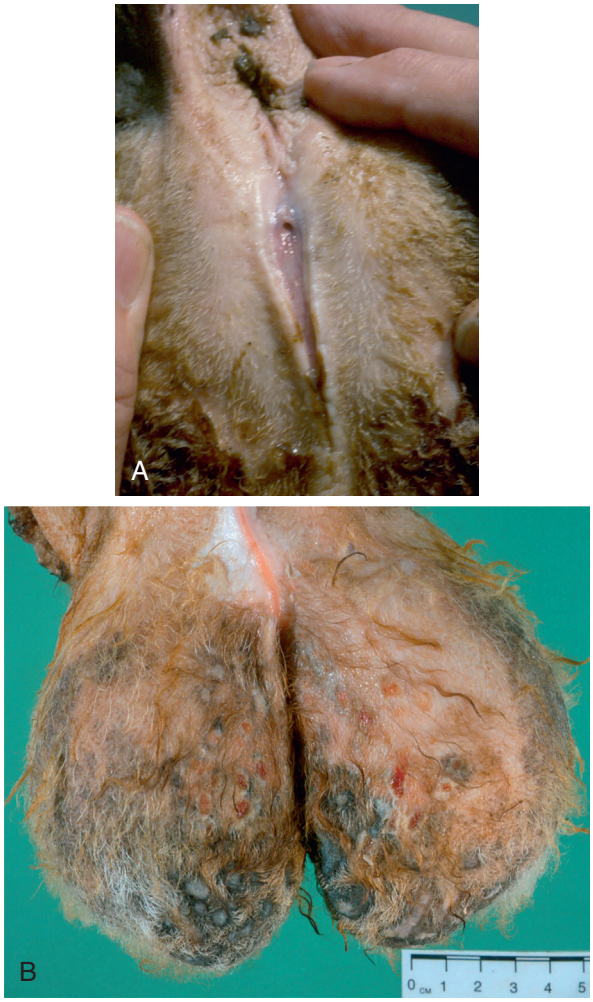
In the mildest cases, the genitalia appear normal, except for an abnormally sited opening of the urethra on the glans penis (Ader & Hobson 1978, Saunders & Ladds 1978). This corresponds to the classification of McFarland (1958) as a balanitic hypospadias. McFarland described three further clinical manifestations of hypospadias: (a) penile, in which the urethra opens ventrally, caudal to the glans penis and cranial to the scrotum; (b) scrotal, in which the urethra opens at the scrotum or between the left and right side of a divided scrotum; and (c) perineal, in which the urethra opens on the perineum. In the perineal form (Fig. 36.15A) the urethral orifice appears to resemble a vulva and, because such an animal also has a scrotum and penis, it may be mistaken for a hermaphrodite. The presence of a cleft scrotum (see Fig. 36.15B) is often associated with hypospadias, presumably as the presence of the urethral defect prevents the two halves of the scrotum from fusing.



• **Fig. 36.15** Hyposapdias in the ram. (A) Perineal hypospadias in a 6-month-old lamb. The urethral folds have failed to fuse over a wide area of the perineum, resulting in a large urinary meatus; the urethra is visible near the dorsal border of the meatus. (B) Incomplete separation of the scrotum in a hogget with perineal hypospadias; the mucosa is exposed above the scrotum and between the scrotum and anus, and the scrotum is severely ulcerated by urine scalding. (With permission from Smith et al. 2006.)

Lesions of the Prepuce Preventing Normal Erection

Adhesions between the peripenile tissues can arise from localised trauma, lacerations, and/or abscessation of the prepuce. Infection of the penis (balanitis) or prepuce (posthitis) is not only painful, causing unwillingness to copulate, but also can result in development of adhesions between the two, preventing protrusion of the penis.

Preputial Eversion and Trauma

Trauma occurs to the prepuce when the mucosa is not maintained within the sheath or as a result of breeding injury. Intermittent protrusion of varying lengths of preputial mucosa is a normal occurrence in many bulls, especially during sexual arousal, and in polled *Bos taurus* bulls (Long et al. 1970, Ashdown & Pearson 1973b) and *B. indicus* bulls with excessively pendulous sheaths. Aplasia or hypoplasia of the retractor muscles of the prepuce (Long 1969) predisposes *B. taurus* bulls to preputial eversion.

Laceration of the prepuce of *B. taurus* breeds does not usually lead to preputial prolapse (Wolfe 2015b). Hence, minor injuries often heal without complication with only minor superficial scarring visible on the surface epithelium of the prepuce. Damage to the prepuce occurs most commonly in the segment of preputial mucosa that is closest to the preputial orifice and therefore most likely to be everted. In such cases, trauma to the everted prepuce results in inflammation, swelling, and oedema of this tissue (Fig. 36.16A). Conversely, laceration of the prepuce and preputial prolapse occurs commonly in the pendulous sheath and long prepuce of *B. indicus* breeds. In such animals the excess prepuce is forced caudally during breeding and forms a collar at the preputial orifice, which becomes contused, lacerated, or may burst during ejaculation (Wolfe 2015b), causes the prolapse of the prepuce and, with the passage of time, the prolapse can become very large (see Fig. 36.16B). Unless replaced soon after eversion, the mucosa may become permanently prolapsed, with severe and diffuse fibrosis and thickening, often with chronic fissure formation and granulation tissue. Phimosis (i.e., inability to protrude the penis), due to stricture of the damaged prepuce, is more common in *B. taurus* than is paraphimosis (i.e., in which the penis cannot be retracted into the sheath). The development of fibrosis in the damaged prepuce results in gross impairment of penile protrusion.

Acutely affected animals may be treated by cold hosing and compression bandaging of the prolapsed prepuce to reduce swelling, disinfectant lavage and debridement, manual replacement of the everted preputial tissue, bandaging of the preputial orifice and insertion of a urine drainage tube, and, often, application of a sling to elevate the sheath against the abdominal wall (Fig. 36.17) (Walker & Vaughan 1980, Wolfe 2015b). However, chronically inflamed tissue generally requires surgical removal, with the objective of restoring free movement of the preputial mucosa during penile erection. The fibrosed preputial tissue is removed by either submucosal resection (Fig. 36.18A and B) or amputation of the prolapse if the fibrotic change extends deeply into the submucosal tissues. In principle, a circumferential incision is made in the outer layer of preputial mucosa, after which the dissection is then deepened so as to excise all the fibrotic tissue before the inner mucous membrane of the prepuce is cut. The removal of the submucosal lesions may cause considerable venous bleeding, requiring careful haemostasis. The inner layer of the mucosa is then sectioned in quadrants so that suturing of mucosal frills can be performed without risk of retraction of the inner membrane into the preputial cavity. Interrupted sutures of catgut or polyglycolic acid are suitable. Resection of lesions around the preputial orifice inevitably reduces the effective length of the penis at erection, so the amount of tissue removed should be limited to the minimum necessary to restore penile movement. Postoperative oedema may

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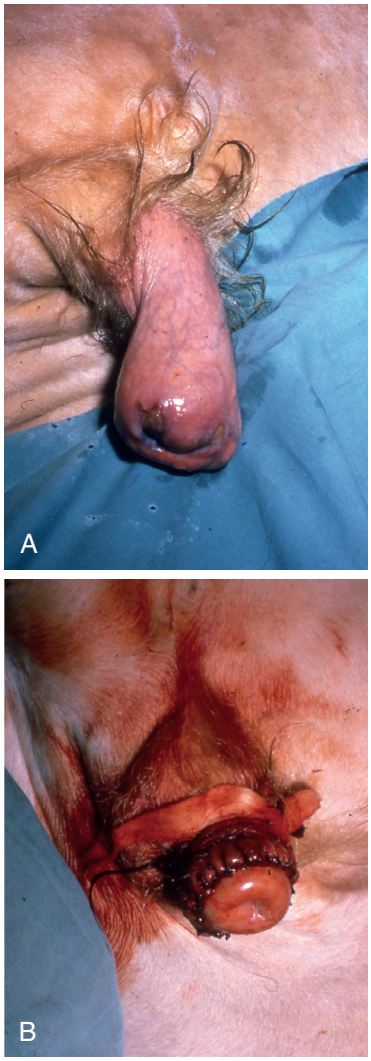
• **Fig. 36.16** (A) Trauma to the prepuce of a *Bos taurus* bull with an excessively pendulous prepuce. (Courtesy Dr Jakob Malmo.) (B) Large prepuce which has become severe with diffuse fibrosis and thickening. (Courtesy Dr Kevin Lawrence.)

• **Fig. 36.17** Application of a sling to elevate a prolapsed prepuce against the body wall. (Courtesy Dr. Kevin Lawrence and Dr. Will Tulley.)

cause temporary protrusion of the sutured tissues, but this soon subsides. After 2 weeks sexual rest, the bull should be teased as frequently as possible until penile protrusion is adequate for intromission. At around 2 months post-surgery the bull should undergo a serving ability test.



• **Fig. 36.18** (A) Chronic fibrosis in a prolapse of the distal prepuce in a Hereford bull. (B) Final repair of the lesion.

Preputial Adhesions

In the bull, preputial adhesions may occur at the site of the reflection of the preputial mucosa on to the penile integument (Fig. 36.19A). This site is less common than at the preputial orifice but presents greater difficulty in treatment. The cause of such lesions is not always clear, but may result from partial or complete avulsion of the prepuce from the penis during copulation or AV collection of semen. Alternatively, lesions may occur when the prepuce becomes lacerated deep within the preputial cavity, also as a result of the ventral prepuce bursting during the ejaculatory thrust. The site of such lesions is not always clear on clinical examination, for the changing relationships between penis and prepuce make it difficult to correlate the apparent site of a lesion with its position when the penis is protruded under anaesthesia. Intrapreputial lesions present difficulties of exposure for surgery. Even under general anaesthesia, it may be difficult to expose the penis fully without tearing the tissues at the site of fibrotic change. After the penis is extruded, forcibly if necessary (see Fig. 36.19B), all fibrotic tissue masses are excised down to the tunica albuginea between two circumferential mucosal incisions at the proximal and distal extremities of the lesion. The mucosal edges are sutured as for distal resections, and the animals are managed similarly in

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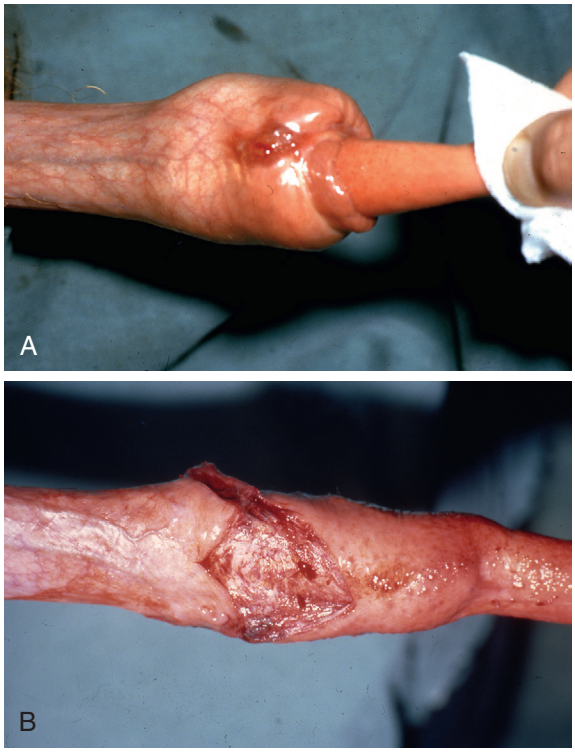

• **Fig. 36.19** Adhesions between the bovine penis and prepuce (A) at the site of the preputial reflections. (B) The adhesions shown in (A) have been forcibly torn apart as a precursor to resection of the fibrotic tissues.

the postoperative period. Such surgery is surprisingly effective, although somewhat unpredictable. Much depends on the length of mucosa that requires resection.

Phimosis

Phimosis indicates a stricture of the preputial orifice that prevents the penis from being protruded. It has been recorded in most of the domestic species.

In dogs, phimosis may be a congenital or acquired defect. Congenital forms are overrepresented in the German Shepherd and Golden Retriever breeds; aetiologies include relatively undersized preputial opening, a small or short penis, and the persistence of tissue bands that connect the penis to the preputial mucosa. Severely congenitally-affected puppies may be unable to urinate adequately, with the consequential balanoposthitis leading to septicaemia and death (Volkman 2013). Acquired lesions include strictures due to fibrosis and stenosis of the preputial orifice, balanoposthitis that causes adhesions between the penis and prepuce, and neoplasia. Balanoposthitis can also occur secondarily to dysuria. When dogs are affected by congenital lesions or a simple stricture of the preputial orifice, they may be treated by removing a wedge of preputial skin, fascia, and mucosa from just behind the ventral aspect of the preputial orifice. Mucosa and skin are then sutured together. Mild urine scalding may occur after surgery, as urine does not run away freely (Burke 1986, Allen 1992). The prognosis for survival of the animal is good; however, the prognosis for use as a breeding sire is poor, and animals that have a congenital form of the condition should not be used for breeding due to the risk of inheritance of the condition.

In the stallion, phimosis is primarily caused by congenital or acquired stricture of the preputial orifice or in neglected cases of penile carcinoma. As in the dog, the presence of the stricture can cause retention of urine within the prepuce, resulting in a secondary balanoposthitis. Congenital causes are virtually unknown in the stallion. Treatment is primarily by removal of a wedge of tissue from the ventral aspect of the prepuce.

Phimosis is not a common condition of the bull; its most frequent aetiology is large penile fibropapillomata that prevent protrusion of the penis. It can also occur as a result of preputial adhesions, especially those due to bovine herpesvirus-1 (BoHV-1)-induced balanoposthitis.

Paraphimosis

Inability to withdraw the penis into the prepuce results from congenital or acquired strictures of the prepuce, paralysis of the penis, and, occasionally, balanoposthitis. Although not strictly constituting paraphimosis, some coital injuries to the penis also prevent its return to the prepuce. The condition is most common in the dog and the stallion but is also reported occasionally in most domestic species.

Paraphimosis in the dog is relatively common after copulation or spontaneous erection. It may also occur when the preputial opening becomes constricted, preventing return of the penis to the prepuce (Kutzler 2013a) or as a sequel to fracture of the os penis, balanoposthitis, or penile neoplasias (Root Kustritz 2001). In cases that have occurred recently, the size of the penis (oedema and inflammation) can be reduced with using cold compression bandages, massage with topical hyperosmotic solutions, and systemic antiinflammatory therapy (Root Kustritz 2001), after which the penis can often be returned to the prepuce with careful manipulation and plentiful lubrication. However, in cases in which the problem is not detected quickly, the penis becomes oedematous and swollen, and the integument increasingly friable. In such cases the preputial orifice may need to be surgically enlarged before the penis can be replaced (Kutzler 2013a). In serious cases or in animals in which the penis has become strangulated, penile amputation may be required, although phallopexy has been successfully employed as a means of managing recurrent paraphimosis in the dog (Somerville & Anderson 2001). The prognosis for cases that have not been treated promptly is therefore guarded, depending on the severity of trauma and the degree of necrosis the penis has sustained. Unfortunately, the dorsal nerves of the penis are highly susceptible to ischaemic damage, and as these are required for the ejaculation reflex, inability to ejaculate is a relatively common sequel of relatively minor penile damage. Castration is not recommended as a long-term treatment for paraphimosis, as the condition also occurs in neutered dogs. However, Root Kustritz (2001) suggested that progestogen therapy may be beneficial in castrates with recurrent paraphimosis.

In the stallion, paraphimosis (Fig. 36.20A and B) usually occurs as a consequence of genital trauma, such as covering injuries, preputial laceration, penile hematoma, castration, inguinal surgery (Schumacher 2012), or other conditions that result in local or systemic oedema. It has also been associated with damage to the innervation of the penis, including disorders of the pudendal nerve or central nervous system. Paraphimosis can also be the sequel of priapism, severe systemic illness, debilitation, and exhaustion. A transient penile paralysis occurs after the administration of phenothiazide tranquilisers (Pearson & Weaver 1978, Lucke & Sansom 1979), which can progress to an irreversible paraphimosis. Paraphimosis has occasionally been reported as a consequence of malicious injury.

Schumacher (2012) described the pathology of the condition. Paraphimosis occurs primarily as the result of oedema collecting

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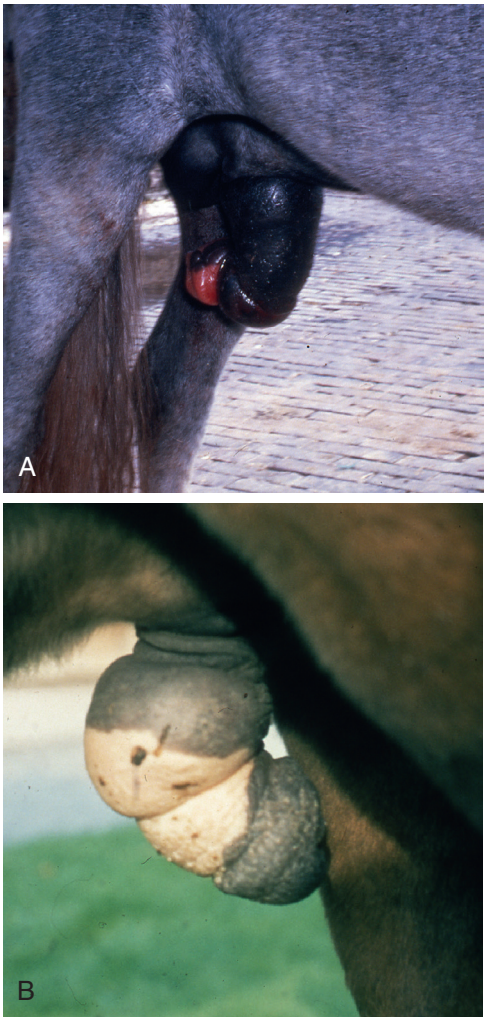

• **Fig. 36.20** (A) Penile prolapse in a horse after being kicked during mating. (B) Priapism, 9 days after administration of neuroleptanalgesia for castration.

in the loose connective tissue between the penis and the internal lamina of the prepuce. The weight of that oedema overcomes the tone of the retractor penis muscle, which is followed by protrusion of the penis and preputial tissue from the preputial orifice. Prolonged protrusion causes further oedema of the penis and prepuce by impairing venous and lymphatic drainage and by the preputial ring becoming a constricting cuff to the venous return from the prolapsed tissues. Additionally, the blood clots in the CCP within 2 to 5 hours of protrusion, making the penis relatively rigid. Subsequently, the penile and preputial epithelium becomes excoriated and then becomes inflamed due to bacterial invasion of the damaged tissue. Eventually, fibrosis of the penile integument and fascia occurs, causing permanent impairment of the normal telescoping action of the prepuce.

The earlier treatment is instigated, the greater the chance of obtaining a satisfactory outcome. Treatment (Cox 1982, Schumacher & Varner 2011) must aim to reduce oedema, prevent trauma to the penile integument, and provide support for the penis until it can be returned to the prepuce. In the early stages, oedema may be dissipated by the use of cold water hosing, cold packs, and compression bandaging, and the use of antiinflammatory drugs and diuretics may also be helpful. Perhaps the most important aspect of treatment is providing adequate support for the penis; drainage of fluid from the penis is greatly facilitated by reducing the tension on the lymphatics that is caused by the weight of the swollen, pendulous penis. Effective supports have been made out of nylon stocking material or U-section plastic guttering, appropriately slung around the horse's hindquarters. The surface of the penis must be protected by the use of ointments that prevent drying or by antiseptic ointments if open wounds are present. Incision of the preputial ring can sometimes be beneficial. Once fibrosis of the preputial tissue has occurred, the prognosis for recovering the animal as a breeding stallion is very poor (Schumacher 2012).

Priapism

Priapism is an uncommon disorder in male cats and dogs and in stallions. Priapism is characterised by a persistent penile erection that lasts for more than a short period of time (variously categorised as between 1 and 4 hours), resulting in pain and dysuria. Priapism is categorised into nonischaemic (arterial aetiology: 'high flow' priapism), which can occur as a result of abnormal blood flow to the penis (e.g., the presence of arteriovenous anastomoses or dysfunction of the neural control of erection) or ischaemic priapism (venoobstructive aetiology: 'low flow' priapism), which can occur due to decreased venous outflow consequent upon occlusive thromboembolic or compressive masses (e.g., tumours or abscess) within the pelvic cavity. The most common cause is trauma that occurs during mating or surgery. Sequelae of priapism are necrosis of the penis and, if it also causes obstruction of the urethra, urine retention/uraemia (Lavely 2009, Kutzler 2013b).

In the dog, the condition can be managed medically or surgically. Medical treatment consists of various combinations of cold water compresses, topical hypertonic solutions, penile lubrication, antiinflammatories, broad spectrum antibiotics, and diuretics (Kutzler 2013b), together with oral pseudoephedrine. Anticholinergic agents may also be of benefit. Surgical treatment consists of replacing the penis within the prepuce by enlargement of the preputial orifice. However, this does not necessarily resolve the congestion of the penis, which may need to be relieved by surgical drainage of the blood from the CCP. Because necrosis of the penis often follows priapism, even when the penis has been replaced into the sheath, amputation is often the final outcome of the condition.

Priapism in the stallion is almost invariably of the 'low flow' type. The most common aetiology is a sequel to the administration of a phenothiazine-derivative tranquiliser; their mode of action appears to be blocking α–adrenergic impulses that mediate detumescence. Rare causes of priapism include general anaesthesia, nematodiasis of the spinal cord, and neoplasia of the pelvic canal (Schumacher 2012). Management is similar to that of paraphimosis, especially in established cases. However, when a failure of detumescence is considered as the primary aetiology, intravenous administration of the cholinergic blocker benzatropine mesylate, plus adrenaline or phenylephrine injection into the CCP, may be beneficial in recently affected animals (Schumacher & Varner 2007). Surgical drainage or the creation of vascular shunts between the CCP and CSP has also been suggested in cases that are unresponsive to medical treatment; penile amputation is a very common outcome in cases of both paraphimosis and priapism.

Other Conditions of the Penis

Strangulation and Necrosis of the Penis

Strangulation may occur as a consequence of paraphimosis or as a result of constriction of the penis by a ring of hair from the preputial orifice or maliciously placed objects. It is most common in long-haired breeds of dog and long-woolled breeds of sheep

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• **Fig. 36.21** Strangulation of the penis of a ram after long strands of fleece became wrapped around the glans during successive copulations.

(Fig. 36.21) but has also been reported in bulls. As with paraphimosis, the prognosis depends on the duration of the vascular constriction and the degree of necrosis that has ensued. Restoration of penile anatomy is relatively easy to achieve for, providing gross necrosis has not occurred, the ability of the organ to heal is relatively good. However, loss of function, particularly impairment of the ejaculation reflex, occurs after relatively short periods of ischaemia. Therefore the prognosis should always be guarded in the first instance. When gross necrosis has occurred, amputation of the penis may be indicated.

Urolithiasis

Urolithiasis is a relatively common condition of castrated male ruminants and of intact rams and bucks (male goats). The presence of urethral calculi causes obstruction of the urethra, which can then lead to necrosis of the penis, rupture of the urethra, rupture of the bladder, tissue necrosis due to urine accumulation, and death from uraemia/toxaemia. The most common predisposing factor is rapid growth of yearlings/2-year-olds on a high concentrate diet that is also rich in magnesium, phosphorous, and/or calcium. Urolithiasis can, however, occasionally occur in any male ruminant, even in the absence of obvious predisposing factors. The most common type of calculus is struvite (magnesium ammonium phosphate); and calcium hydrogen phosphate dehydrate; silicate and calcium carbonate calculi can also occur.

In the early stages of the disease, animals may frequently attempt to urinate, but, as the condition worsens, the primary signs become anorexia and abdominal pain. On clinical examination, it may be possible to palpate a large intact bladder, or, if the bladder has ruptured, there will be a large volume of fluid (urine) within the abdomen. If the urethra ruptures, there will be progressive fluid accumulation within the ventral body wall. Rupture of bladder or urethra is accompanied by reduced signs of abdominal pain and increased signs of uraemia (Van Metre 2004).

The most common site of primary blockage of the urethra of rams and bucks is the base of the vermiform appendage. If the animal is presented in the early stages of the disease, it can be resolved by simply amputating the appendage. Recovery is rapid and fertility will not be impaired. However, it is not uncommon for calculi to completely obstruct the urethra leading to varying degrees of penile necrosis and rupture of the urethra with infiltration of urine into the surrounding sheath. In valuable animals, tube cystotomy is the preferred approach, as the alternative typically involves perineal urethrostomy, accompanied, if necessary, by penile amputation.

Cattle are also affected by urethral calculi, of which the most common forms are silicates, xanthine, and different types of carbonates and phosphates. Phosphate calculi are found mainly in feedlot cattle, whereas silicate calculi occur predominantly in grazing cattle. The site of obstruction in cattle is usually the distal sigmoid flexure, so the only feasible means of treatment is by perineal urethrostomy (Vermunt et al. 2010).

Prevention is by controlling the mineral content of the diet, feeding salt to stimulate thirst, ensuring that animals have an adequate supply of water, and adding dietary supplements to the ration to acidify the urine.

Fracture of the Os Penis

This is a relatively common problem in the dog. Clinical signs may be evident as an immediate consequence of the fracture or may present later as a cause of penile abnormalities or failure of coitus. The condition is sometimes associated with trauma to the penis, but the aetiology is often obscure (Johnston et al. 2001). Manifestations of the condition range from dysuria and haematuria through to abnormalities of erection and genital pain to renal failure. Simple acute fractures may be managed conservatively via closed reduction and placement of an indwelling urinary catheter to impart stability during healing. Long-standing fractures, particularly where callus formation causes dysuria due to constriction of the urethra, require more radical surgical intervention such as removal of the os penis or penile amputation (Voelkl 2013).

Miscellaneous Conditions

Complete avulsion of the preputial mucosa from its attachment to the glans penis is an occasional injury of bulls that usually results from careless use of an AV (Roberts 1986) but may occur in naturally mated bulls.

Diphallus, or duplication of the penis, is an exceedingly rare condition but has been occasionally reported in the bull and dog. Lesions may be confined to the penis, or there may be more generalised urogenital or skeletal abnormalities.

Disuse atrophy of the retractor penis muscle can occasionally occur in bulls that have been unable to achieve an erection over a long period of time. Although calcification of the retractor penis muscle, which occurs in aged bulls, does not affect mating ability, there is some evidence it may increase the risk of preputial eversion, one of the major predisposing factors for prolapse of the prepuce.

Balanoposthitis

Infections of the penis and prepuce are common in the dog, bull, and ram, occasional in the stallion, and rare in the boar and cat. Low grade infection of the preputial cavity is very common, and rarely causes clinical disease. Severe balanoposthitis can cause pain, unwillingness to mate, preputial stenosis, adhesions between penis and prepuce, and peripenile adhesions.

Balanoposthitis in the Dog

Mild balanoposthitis is particularly common in the dog, in which the prepuce almost always contains a mild seropurulent exudate, which is rarely indicative of clinical disease. It does cause a great deal of anxiety to some dog owners, who find both the discharge of such material from the preputial orifice and their dog's efforts to cleanse himself of it offensive. Prophylactic treatment of these mild infections is also frequently requested by owners of stud dogs. Such requests should be considered with some caution for, whereas the use of mild antiseptic douches or bland antibiotic or antiseptic ointments rapidly clear any infection, the loss of the

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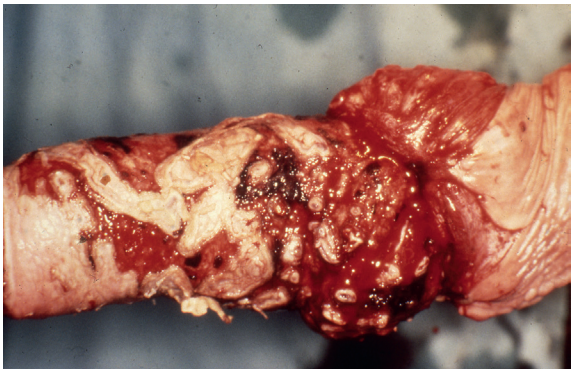

• **Fig. 36.22** Acute balanoposthitis in the bull, caused by BoHV-1 infection.

• **Fig. 36.23** Severe adhesions between most of the free part of the penis and the prepuce, as a consequence of healed lesions after BoHV-1 infection.

normal bacteriological flora from the preputial cavity can predispose to the establishment of opportunist infections by organisms of clinical significance.

More serious cases of balanoposthitis can occur in conjunction with opportunistic bacteria (particularly *Escherichia coli*), mycoplasmas (chiefly *Mycoplasma canis*), and viral infections with canine herpesvirus or, occasionally, calicivirus (Johnston et al. 2001). It can also occur in animals with atopic dermatitis (Root Kustritz 2001). Clinical signs include purulent preputial discharge, which may also be bloody, accompanied by inflammation and ulceration of the penile integument, conspicuous lymphoid nodules, and, sometimes, petechial or submucosal haemorrhages (Johnston et al. 2001). Treatment is by infusion of bovine intramammary formulations of appropriate antibiotics and flushing of the preputial cavity with water or saline.

Bovine Herpesvirus

In bulls, a severe, ulcerative balanoposthitis is caused by the genital form of BoHV-1 infection (McEntee 1990, Weiblen et al. 1992). The penile integument and preputial mucosa are initially covered with small necrotic foci, which develop into deep ulcers. In severe cases, these coalesce into large areas over which the penile integument has sloughed. Secondary bacterial infection of the ulcers results in a severe, purulent balanoposthitis, causing very considerable discomfort to the bull. Affected bulls may exhibit swelling and pain in the region of the penis and are commonly dysuric. There is characteristically a fetid, watery, preputial discharge, containing shreds of sloughed tissue and small amounts of fresh blood (Fig. 36.22). Cows that have been served by affected bulls may develop signs of infectious pustular vulvovaginitis; the bull may be relatively asymptomatic despite causing characteristic lesions of pustular vulvovaginitis in the cows (e.g., Pritchard et al. 1997). It is unusual (although not unknown) for animals with genital lesions of BoHV-1 to also show upper respiratory (infectious bovine rhinotracheitis) signs.

Mild cases of BoHV-1 balanoposthitis may heal without complication over 1 to 2 weeks, but more severe cases can develop adhesions between the penis and prepuce. Adhesions develop quickly, appearing within only about a couple of weeks of the onset of acute symptoms. Adhesions can be extensive, involving most of the free part of the penis (Fig. 36.23). Affected bulls are unable to achieve a normal erection, with only a few centimetres of the tip of the penis being protruded through the preputial orifice.

BoHV-1 is transmissible in semen, which presents considerable risk if bulls that either have active infection or are seropositive are used in AI programmes. For this reason, many regulatory authorities preclude the use of seropositive bulls as semen donors or insist on testing the semen for BoHV-1 by means of viral culture or polymerase chain reaction (OIE 2017a).

Penile Granulomas in Bulls

Bulls are also susceptible to granuloma formation on the penis, which is a nontransmissible and usually asymptomatic condition (Cox 1982, Roberts 1986). Occasionally, it causes pain and an unwillingness to mate. The condition is characterised by hypertrophy of the lymphoid nodules of the penis in the absence of an obvious, purulent balanoposthitis. Sexual rest and prophylactic infusions of oily suspensions of antibiotic into the prepuce usually produces a resolution of clinical signs. However, granuloma of the penile integument of bulls is also associated with *Ureaplasma diversum* infections (Marques et al. 2009), which are a potentially serious infectious cause of infertility and/or abortion. Tuberculous balanoposthitis has been described in areas where the disease is epizootic (Williams 1943). Its signs include enlarged, granulomatous lesions of the penis that may bleed, peripenile adhesions, and secondary phimosis. This condition must be differentiated from actinomycosis of the prepuce, which causes a similar syndrome.

Ulcerative Posthitis ('Pizzle Rot') of Rams

Balanoposthitis is a relatively common condition of male sheep, in which it is colloquially known as pizzle rot. Bacterial balanoposthitis is caused by a number of organisms, of which *Corynebacterium renale,* an organism that grows readily in alkaline urine, is the most important. The disease is more common in wethers than in rams because of the effects of early castration on development of the genitalia and the absence of circulating testosterone. The condition is most common in animals that are fed on lush pastures that contain high levels of rumen degradable protein. From the urea contained in the urine, the bacteria produce ammonia, which scalds the preputial region. Ulcers and scabs develop around the preputial orifice. Eventually, the preputial orifice may become occluded, leading to urine retention within the preputial cavity (Fig. 36.24). Treatment of advanced stages of the disease is seldom successful, but mild lesions resolve simply by reducing the urea load in the urine by restricting access to lush grass (West et al. 2018) and shearing the underside of the ram. The condition can be prevented in intact rams by keeping the belly and preputial region shorn. In wethers, especially the older animals that are kept for wool rather than for meat, testosterone implants are used prophylactically, with considerable success.

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• **Fig. 36.24** Balanoposthitis in the ram. Characteristic staining of wool around the preputial orifice. (With permission from Bruere & West 1993.)

• **Fig. 36.25** Scars left on the ovine penis by healed contagious pustular dermatitis (orf) lesions.

The same condition occurs in young bulls fed high protein rations or pasture in preparation for sale. The preputial orifice of affected bulls should be debrided followed by topical application of intramammary preparations of penicillin or cloxacillin, with more severe cases also being treated with parenteral penicillin.

Contagious Pustular Dermatitis Affecting the Genitalia of Rams and Bucks

Lesions due to contagious pustular dermatitis (*syn: orf, scabby mouth*) virus occur around the genitalia of rams and bucks – on the preputial orifice, prepuce, and glans penis – where the shallow ulcers are generally covered by a scab. There may also be lesions present in the other sites characteristic of orf, namely the lips, nostril, and feet and in females of the same flock, the vulva and teats. The disease is spread by contact, including venereal contact. Affected rams are generally unwilling to achieve intromission, but their libido is often maintained sufficiently to allow mounting to continue. Thus, the condition may escape notice for quite long periods of time until it is realised that there is a very high incidence of returns to oestrus among the ewes. In these situations the primary lesions are often healed by the time animals are presented for clinical examination with little evidence, other than the scars left on the glans penis by the healing ulcers (Fig. 36.25), that infection has occurred.

Dourine

The most serious cause of balanoposthitis in the stallion is the notifiable disease of dourine (De Vries 1993, Luckins et al. 2004). Dourine is caused by the protozoan parasite *Trypanosoma equiperdum* and occurs in the Middle East, North Africa and South America. Pockets of infection also occur in the Balkans and South Africa. The infection is the only trypanosomosis that is not transmitted by an invertebrate vector, and there is no known natural reservoir of the parasite other than infected equids (OIE 2017b). Infection is predominantly venereal, but can also be transmitted through infected AI equipment, and can be passed to foals through vaginal discharges from the mare. It affects all domestic equids, but donkeys and mules are more resistant than horses.

The incubation period, severity, and duration of the disease vary considerably. It is often fatal, but there are records of spontaneous recoveries and of latent carriers. The disease is marked by stages of exacerbation, tolerance, or relapse, which vary in duration and which may occur once or several times before death or recovery (OIE 2017b). The initial sign is oedema of the prepuce, penis, scrotum, and surrounding skin, which may be sufficiently severe to cause paraphimosis. Inguinal lymph nodes are often enlarged, and there may be a mucopurulent urethral discharge. There are also neurological signs of incoordination and facial paralysis, and a fluctuating pyrexia. A pathognomonic sign, if present, is the presence of 5 to 8 cm oedematous plaques in the skin, usually over the ribs. Death may occur within 1 to 2 months of infection (although in southern Africa it is more common for the condition to be more chronic) as the consequence of vascular degeneration causing peripheral nerve degeneration, muscle wasting, emaciation, and paralysis. The condition can be treated in its early stages by trypanocidal drugs, although in many countries (i.e., northern and western Europe, the United States, Canada, and Australasia) control is through notification and slaughter policies.

Equine Habronemiasis

In parts of the world where *Habronema muscae* occurs, larval infection produces granulomas in the equine penile and preputial integument. Cutaneous habronemiasis (*syn: granular dermatitis*) is a granulomatous, mildly pruritic disease caused by cutaneous migration and encystment of the larvae of the equine stomach worm *Habronema.* Larvae passed in the faeces are ingested by fly maggots, and after the maggot pupates, the larvae are deposited on wounds from the feeding fly. The disease is associated with the seasons in which flies are abundant and, in temperate climates, lesions commonly regress during the winter (Schumacher 2012).

Genital lesions are most commonly found at the preputial ring and the urethral process, where larval infestation causes local hypersensitivity and extensive granulomatous reactions. These bleed easily, may appear ulcerated, cause pruritus, and can cause prolapse of the affected tissue from the preputial orifice. Lesions of the urethral process can result in dysuria (Stick 1981). Treatment is by eliminating the larvae with systemic insecticides (e.g., ivermectin) or diethylcarbamazine and reducing the hypersensitivity reaction (e.g., prednisolone) (Schumacher & Varner 2011). Occasionally, surgery is required to relieve urethral obstruction or to remove the scar tissue that may form during healing (Stick 1981).

Equine Coital Exanthema

A more common condition of stallions is equine coital exanthema, or horse pox (reviewed by Barrandeguy & Thiry 2012), which is

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caused by equine herpesvirus-3 (EHV-3). The mode of transmission is predominantly venereal, which includes coitus, via a contaminated artificial vagina and through insemination with infected semen. It may also be transmitted by insect vectors (Gibbs et al. 1972) or fomites, although non-coital transmission has also been associated with the genitonasal contact via nuzzling. In common with many other herpes viruses, EHV-3 can become latent and undergo periods of reactivation (Barrandeguy et al. 2008), such that stallions can become long-term carriers and/or excreters of the virus.

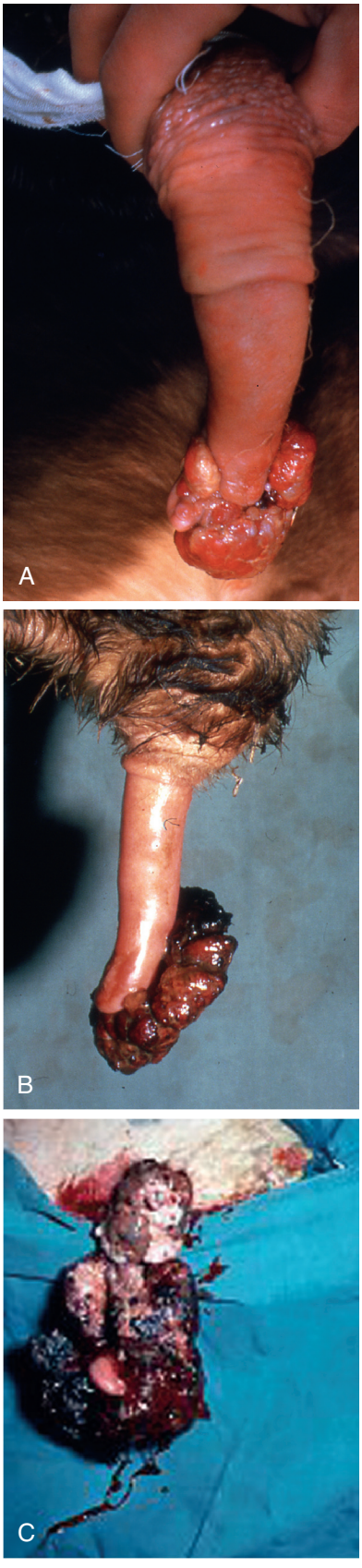
Lesions occur mainly on the free part of the penis and prepuce. After serving an infected mare, small papules initially appear that, within a few days, develop into vesicles surrounded by hyperaemia and oedema. These rupture and form shallow, raw, or encrusted erosions or ulcers. Uncomplicated lesions heal within 10 to 14 days (Blanchard et al. 1992). When the density of lesions is sufficiently great, large ulcerated areas may appear. Mild infections do not impair libido, although lesions with severe secondary infection may do so. Blood may be present in the ejaculate (Ley & Slusher 2007). Diagnosis is by detection of the virus or demonstration of seroconversion. Control is achieved by preventing clinically affected animals from mating, although individual animals may need affected areas to be washed weekly with antiseptic solution to limit the establishment of secondary infection. Immunity is, however, short term, so reinfection is a distinct possibility.

Penile Neoplasia

Bull

The only common tumour of the bovine penis is the virally induced fibropapilloma. Young cattle commonly have papillomata of the skin, alimentary tract, and mucocutaneous junctions, including the genitalia. The penile integument, particularly its terminal 5 cm, is a common site for such tumours, which may be single or multiple, sessile or pedunculated (Fig. 36.26). Tumours can be found in intact and castrated animals but rarely persist beyond 3 years of age. Clinical effects vary according to the size and the morphology of the lesions. Haemorrhage and ulceration of the tumour mass caused by mating are the most common sequelae, and the associated pain is sometimes sufficiently severe to impair libido. Very large lesions can sometimes result in intermittent or complete failure of retraction of the protruded penis into the sheath, with subsequent marked to severe inflammation of the prolapsed penis. Rarely, rapid growth of fibropapilloma within the preputial cavity can result in compression and occasionally rupture of the urethra.

Fibropapillomas sometimes undergo necrosis with varying degrees of sloughing; others become detached during coitus, whereas others spontaneously regress. However, such resolutions cannot be predicted, so recourse to surgery is frequently indicated (Pearson 1972). Single, pedunculated lesions can be ligated relatively easily during electroejaculation of bulls, but excision of sessile lesions requires sedation and either local infiltration of local anaesthetic or administration of a bilateral pudendal nerve block (Morgan 2007). Very occasionally, removal of large tumours that envelop the glans penis requires general anaesthesia. Although haemorrhage from the site of removal may persist for some time, it is rarely dangerous, and, provided the tumour does not recur, the wounds generally heal quickly. Laser surgery and cryosurgery have also been used in the removal of tumours. Regardless of the method of analgesia or surgery, care must be exercised to avoid incising the urethra as its highly vascular mucosa may continue to bleed at ejaculation for long periods afterwards. Hence, catheterisation of the penile urethra is recommended if there is any risk of its involvement at the base of the tumour.



• **Fig. 36.26** Penile fibropapillomata in the bull. (A) Lesions exposed in conscious bull by means of pudendal nerve block. (B) Lesion covering an extensive area of the tip of the penis. (C) Massive tumour causing penile prolapse, urethral rupture, and localised cellulitis in a 10-month-old castrate.

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Penile fibropapillomas do not metastasise, but a small proportion of tumours exhibit a remarkably aggressive recurrence after surgical removal. There is debate about the incidence of such recurrences, but possibly 10% of lesions recur with sufficient rapidity to be obvious again within 3 to 4 weeks of surgery (Pearson 1977). Administration of an autogenous tissue vaccine markedly reduces the incidence of recurrences (Desmet et al. 1974). Similarly, when clients are not prepared to pay for surgery to remove fibropapillomas, administration of an autogenous vaccine should be considered, as this approach has been shown to be moderately effective. The prognosis for use with breeding animals is generally good, although libido may take some time to recover in animals that have had long-standing, painful, ulcerated lesions. Gilbert and Fubini (2004) considered that, if aggressive recurrence occurs or if papilloma-like lesions are encountered in mature bulls, the lesion may be a malignant fibrosarcoma. This can be confirmed by histopathology. Culling is indicated in such cases.

Stallion

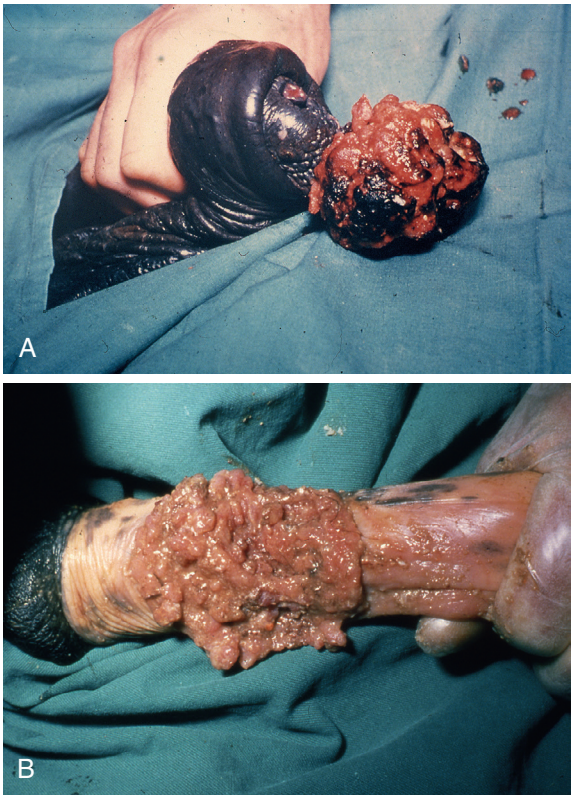
Neoplasia of the equine penis and prepuce are common. Tumours of the penis and prepuce include squamous papilloma, squamous cell carcinoma, sarcoid, melanoma, mastocytoma, and hemangioma (Schumacher 2012). Melanomas are occasionally found on the prepuce of aged grey horses.

Squamous cell carcinoma of the glans penis (Fig. 36.27) or prepuce are the most common genital tumours of the horse. It is believed that squame cell carcinoma develop in response to the presence of smegma in the prepuce. It was originally thought that smegma contained a specific carcinogen (Plaut & Kohn-Speyer 1947), but it is now thought that it is the chronic irritation and balanitis that it causes which induces neoplastic changes (Brinsko 1998). The tumour is therefore encountered most frequently in geldings, particularly in aged animals. Schumacher and Varner (2007) suggest that it is also more common in breeds of horses that lack pigmentation of the genitalia.

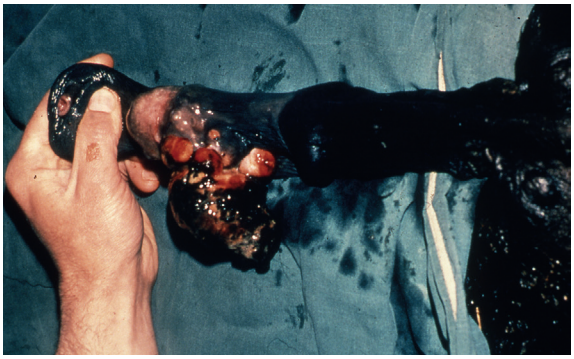
Tumours grow relatively slowly, but eventually progress to become large, ulcerated tissue masses that may cause bloody preputial discharge or penile prolapse. Metastasis is slow, although the tumour does spread within the preputial cavity and, eventually, metastasises to local lymph nodes. However, local lymphadenitis may also occur as a result of the secondary infection that invariably occurs with these tumours, so care must be exercised in giving a prognosis. Occasionally, penile carcinomas are highly invasive and rapidly destroy the body of the penis (see Fig. 36.27B).

Early cases and animals with small tumours can be treated by cryotherapy or by repeated topical applications of fluorouracil to the lesion at 14-day intervals (Fortier & MacHarg 1994). Some lesions of the more loosely attached prepuce can be dealt with by simple or reefing excision. However, more advanced penile carcinomas are best treated by radical amputation of the penis, with an urethrostomy on the penile stump within the sheath or directly to the sheath (Walker & Vaughan 1980). The recurrence rate is relatively high, around 20%, particularly in horses in whom the carcinoma has invaded along the urethra (Mair et al. 2000).

Sarcoids are also diagnosed on the penis, prepuce, and scrotum of entire and castrated horses, donkeys, and other equids. In most circumstances, such lesions can be extirpated without difficulty using a variety of surgical methods (Schumacher & Varner 2011). However, the presence of multiple, recurrent sarcoids on the sheath and prepuce (Fig. 36.28) may necessitate its complete ablation and amputation of the penis (Cox 1982). Grey horses may develop melanomas of the penis or prepuce (Fig. 36.29).



• **Fig. 36.27** Penile and preputial tumours in the stallion. (A) Squamous cell carcinoma of the penis in an aged gelding. (B) Squamous cell carcinoma of the penis associated with tissue infiltration and destruction.



• **Fig. 36.28** Multiple sarcoids of the equine penis and prepuce.

• **Fig. 36.29** Multiple melanomata formation in the prepuce of an aged grey gelding.

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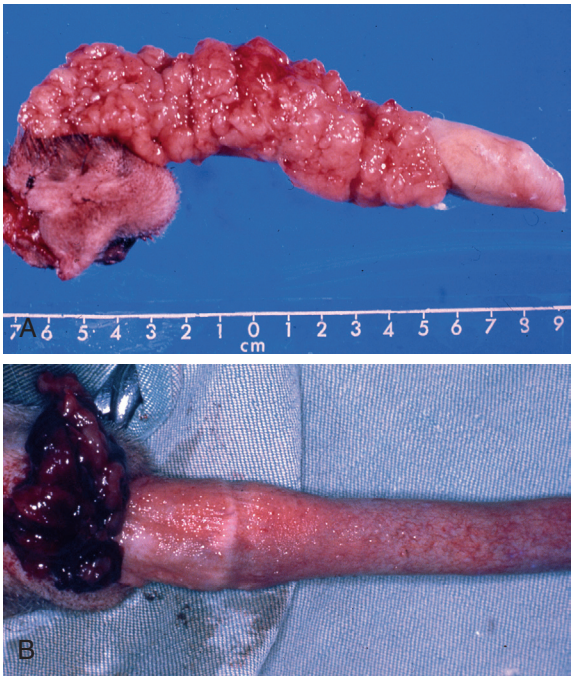
Their progress is generally slow, with only local invasion of tissue, but aggressive lesions that metastasise are also encountered.

Dog

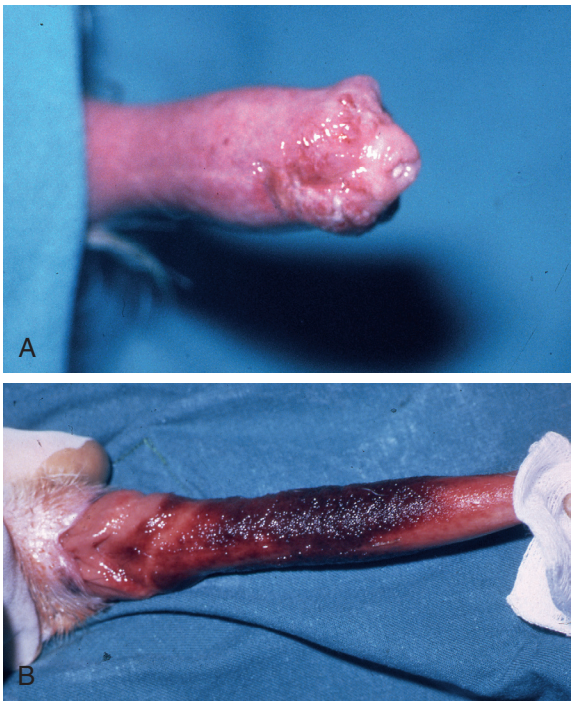
A great many tumours affect the penis and/or prepuce of the dog. Tumours of the penis include lymphosarcoma, hemangiosarcoma, and squamous cell carcinoma; those of the prepuce include mast cell tumour, papilloma, and carcinoma. Chondrosarcoma of the urethra has been reported, and various tumours of the os penis have been described (Voelkl 2013). The most common tumour of the penis and prepuce is the transmissible venereal tumour (Jones & Joshua 1982). Most tumours of the penis present with clinical signs of difficulty of urination, progression to dysuria, and haematuria. There is distension of the bladder, abdominal pain, and enlargement of the preputial area.

Transmissible venereal tumour has been reported in most parts of the world, but is most commonly diagnosed in dogs located in large urban areas. It is transmitted during coitus and, occasionally, by nuzzling of the genitalia. The primary site for these tumours is the external genitalia of both males and females, but lesions also occur around the mouth and nose (Johnston et al. 2001, Levy et al. 2006, Marcos et al. 2006). Transmission appears to be caused by direct implantation of tumous cells that are exfoliated during coitus (Martins et al. 2005). Younger, sexually active animals (< 5 years old) are most often affected, but there is no breed predisposition. Growth is rapid, with clinical signs generally becoming apparent after a short incubation period (typically 5–6 weeks but sometimes considerably less). The rate of metastasis is relatively low, and up to 20% of animals undergo spontaneous remission (Nayak et al. 1987). A fetid, bloody preputial discharge may be present, and, on exteriorisation of the penis, characteristic fleshy, greyish-red, nodular masses are observed (Foster 2012). The tumours ulcerate easily and are friable (Fig. 36.30) and readily bleed when handled. The tumour is locally invasive in about 50% of affected animals, with metastases to local lymph nodes occurring in a small proportion of affected dogs. Rarely, widespread metastases through viscera and nervous tissues have been reported. Treatment, when this is possible, is by removal of the penis and prepuce, but the recurrence rate is relatively high. Alternatively, chemotherapy (e.g., vincristine) and/or radiotherapy (Cohen 1985, Wittrow & Susaneck 1986, Singh et al. 1996) or laser therapy (Kangasniemi et al. 2004) have been used.

Penile fibropapillomas (Fig. 36.31) also occur in the dog. Some lesions are virally induced, whereas others clearly have a non-viral aetiology. Non-viral papillomas, variously called squamous papillomas or idiopathic mucosal penile squamous papillomas, are likely to arise from local trauma or irritation (Cornegliani et al. 2007). Viral papillomas, caused by canine papilloma virus, occur on the penis or preputial mucosa where they first appear as plaque-like or raised papillomatous lesions (Foster 2012). The types of papilloma are clinically indistinguishable from each other but present in a variety of forms; they may appear as pedunculated, cauliflower-like masses arising from the penile mucosa (Voelkl 2013) or can be ulcerative, locally keratinised, and poorly circumscribed. They may also be completely sessile. Such lesions bleed profusely after mating or when the penis becomes erect, but their development may be remarkably slow, even over a number of years. Papillomas can rarely develop into squamous cell carcinomas. Non-neoplastic lesions that can have a very similar appearance include tags, hyperplastic polyps, and excessive granulation tissue (Foster 2012).



• **Fig. 36.30** Transmissible venereal tumour of the dog. (A) Tumour formation over most of the penile integument. The characteristic fleshy, greyish-red, nodular masses of the tumour are readily apparent. (B) Tumour developing in the preputial fornix.



• **Fig. 36.31** Penile papillomata of the dog. (A) Localised lesion. (B) Diffuse, 'florid' lesion.

Conditions Causing Failure of Ejaculation

There are a few conditions in which ejaculation does not occur, despite normal mounting. Such conditions can be broadly divided into those in which the ejaculation reflex is impaired and those in which localised pain makes the animal unwilling to ejaculate.

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The former conditions generally occur when some damage has occurred to the neural pathways between the glans penis and the spinal cord. Strangulation of the penis, with ensuing damage to the sensory dorsal nerve of the penis, causes ejaculation failure, whereas, in older bulls, compression of the spinal nerve roots by age-related exostoses can also preclude ejaculation.

Localised pain is similarly effective in preventing ejaculation. Localised peritonitis in the caudal abdomen of ruminants causes pain during the ejaculatory thrust, so affected animals are often willing to mount but unwilling to complete an ejaculatory thrust. Animals with back pain behave similarly, although they may be less willing to mount. Finally, some painful conditions of the penis, such as orf in rams, make the animal unwilling to achieve intromission and ejaculate, despite their willingness to mount.

Haemospermia

Haemospermia, due to leakage of blood from part of the genital tract, is a significant cause of infertility in stallions and has been described in the bull. Some cases are caused by obvious lesions of the penile integument, such as trauma, neoplasia, or balanoposthitis. Seminal vesiculitis can also be associated with haemospermia, although changed (brownish) blood is more usually associated with this condition, at least in the bull. Profuse haemorrhage, arterial in appearance, has been observed in bulls and stallions at the end of ejaculation, as a result of leakage of blood from the CSP into the lumen of the urethra, typically from a lesion in the caudal/ventral wall of the urethra (Ashdown & Majeed 1978, Schumacher & Varner 2007).

Conditions Causing Failure of Fertilisation

Fertilisation failure, despite normal copulation, generally characterises diseases of the testis (including abnormalities of spermatogenesis), epididymis, and accessory glands. Many of the conditions causing failure of fertilisation can be diagnosed by an examination of the external genitalia of the male, but others can only be diagnosed by semen evaluation.

Moreover, diagnosis of failure of fertilisation on the part of the male animal requires elimination of female factors through examination either of records or of the females themselves. Likewise, causes of fertilisation failure that represent pathologies of the reproductive tract must be differentiated from management factors (e.g., overuse) that adversely affect fertility.

Conditions Affecting the Testis

Orchitis

Orchitis ranges from a mild infection of the testis, scarcely distinguishable from testicular degeneration, through to gross suppurative or necrotic destruction of the organ. Orchitis can arise from a primary infection or by the haematogenous route. In rams, it is likely that many infections become established after fighting injury to the testis. *Brucella* species cause orchitis in many domestic animals: *B. abortus, B. canis, B. melitensis,* and *B. suis* affecting bulls, dogs, sheep and goats, and boars, respectively. Granulomatous orchitis in bulls can potentially be due to tuberculosis (Adeniran et al. 1992). However, the majority of isolates from cases of orchitis are either nonspecific bacteria or mycoplasmas or are the particular pyogenic organism for the animal species (e.g., *Trueperella pyogenes* in cattle). Ascending infection from the urinary tract has been postulated, but haematogenous spread seems more probable.

In dogs, in addition to common bacteria (e.g., *E. coli,* staphylococci, streptococci), *Mycoplasma canis* and canine distemper virus are significant infectious causes of orchitis. Non-infectious causes, particularly autoimmune destruction, are also important in the dog. Autoimmune orchitis may occur in association with disruption of the blood–testis barrier, as part of a generalised autoimmune disorder or from idiopathic causes. However, because of the importance of *B. canis* as an infectious cause of infertility, dogs (in countries where it occurs) with orchitis should routinely be tested for evidence of this infection (Johnston et al. 2001). Orchitis is rare in the cat, with bite wounds as the most common aetiological agent. Feline infectious peritonitis virus can also cause orchitis.

Orchitis is more commonly unilateral than bilateral and may involve the epididymis. During the acute phase of the disease, the affected testis is inflamed, with consequent hyperaemia, heat, and oedema and swelling (Fig. 36.32A and B). The testis may become grossly enlarged – up to two or three times its normal size. The testis is often very painful, so that the animal resents it being palpated. The pain may be sufficiently severe to produce an altered gait and systemic pyrexia may occur. The localised inflammation usually causes temperature-dependent degeneration in the unaffected testis. If the condition progresses to the chronic phase, the testis becomes shrunken, fibrotic, and adherent to the tunic and scrotum. Abscesses may break through the scrotal skin.

Orchitis invariably causes a great deal of destruction of the affected testis, and medical treatment is only likely to be successful when initiated early in the course of the disease. Treatment for at least 10 days with broad spectrum antibiotics (particularly macrolides), nonsteroidal antiinflammatory drugs, and cold hosing is recommended. In advanced cases, there may be large, coalescent abscesses occupying much of the testicular parenchyma (see Fig. 36.32D), or it may be necrotic with most of the seminiferous tissue destroyed. The prognosis for saving the affected testis in cases with obvious severe palpable changes is nil. If it is hoped to salvage an affected animal for breeding, removal of a unilaterally affected testis should therefore be advocated at the earliest possible stage of the disease to limit degeneration of the unaffected testis (see Fig. 36.32C). If bilateral orchitis occurs, the prognosis for future breeding is usually very poor, and castration should be performed as soon as it is safe to do so. Pretreatment with antibiotics may reduce the risk of haematogenous spread of infection during surgery.

Testicular Hypoplasia

Testicular hypoplasia implies an incomplete development of the germinal epithelium of the seminiferous tubules due to inadequate numbers of germinal cells within the testis. Lack of germinal cells may arise through partial or complete failure of the germinal cells to develop in the yolk sac, failure to migrate to the genital ridge, failure to multiply in the developing gonad, or widespread degeneration of embryonic germinal cells within the primitive gonad. Mild cases may exhibit moderate oligospermia or poor sperm morphology, but severe cases may be aspermic.

Sporadic cases of hypoplasia occur in all species, occasionally, but not often, with a clear familial predisposition (e.g., Gunn et al. 1942, Holst 1949, McEntee 1990, Siliart et al. 1993). The condition (Fig. 36.33) is relatively common in rams (West et al. 2018) and bulls, particularly *Bos indicus* genotypes. A hereditary form of hypoplasia exists in Swedish Highland cattle (Lagerlof 1936, 1951, Eriksson 1950), affecting the left testis more commonly than the right. An animal with unilateral testicular hypoplasia is

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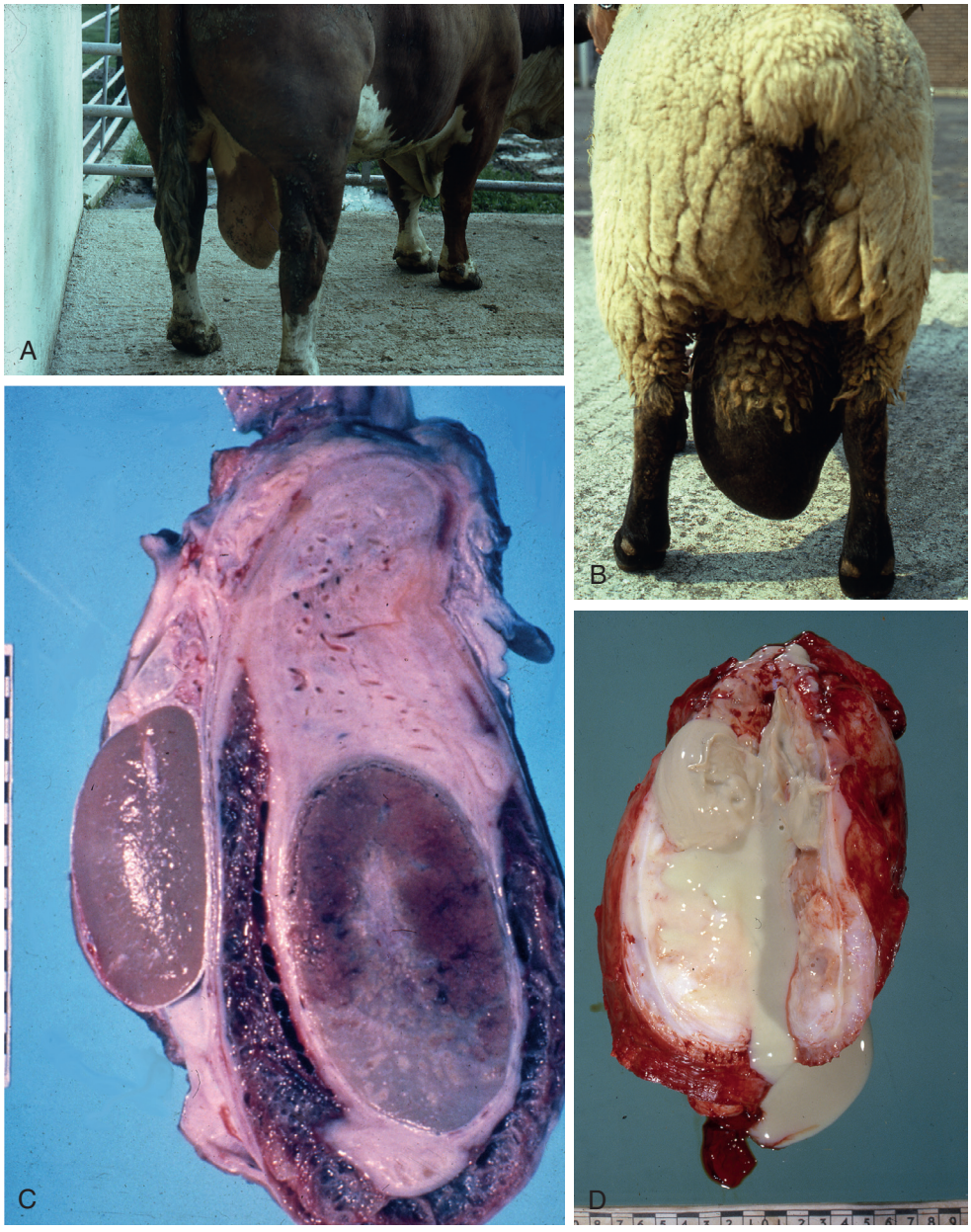

• **Fig. 36.32** Acute orchitis in (A) a Simmental bull and (B) a ram. (C) Longitudinal section of the scrotum of the bull in (A), showing inflammation within the substance of the testis and in the vaginal tunics. Degeneration of the contralateral testis has caused a reduction in its size. (D) The affected testis of the ram in (B) after removal, showing massive abscessation and destruction of testicular parenchyma. ((A) and (C) with permission from Parkinson 1991.)

shown in Fig. 36.33B. Arthur (1975) recorded a high incidence of hypoplasia in the Welsh Mountain pony, in which the right testis was most commonly affected and for which he considered that an inherited aetiology was likely.

Klinefelter's syndrome (karyotype XXY) is a sporadic cause of testicular hypoplasia in bulls (Logue et al. 1979) and has been reported in rams, boars, and dogs (Breeuwsma 1968, Bruere et al. 1969, Clough et al. 1970). It is also particularly associated with male tortoiseshell and calico cats (Smith & Jones 1966, Centerwall & Benirschke 1975, Long et al. 1981). The spermatogonia of such animals fail to develop, so the seminiferous tubules are virtually devoid of spermatogenic cells. The semen of such animals is therefore aspermic, although, as the Leydig cells are unaffected, libido is normal.

Diagnosis of the condition is by measurement of scrotal circumference, which is lower than acceptable limits for the species and breed. Palpation of the testes reveals one or both to be small and flabby, but regular in outline and freely movable in the scrotum. Semen analysis may reveal aspermic or oligospermic ejaculates, sometimes with markedly abnormal morphology or motility characteristics of such sperm as are present. By contrast, libido is generally normal, and, for this reason, the condition may escape the owner's attention until the failure to achieve satisfactory pregnancy rates is noticed.

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• **Fig. 36.33** (A) Testicular hypoplasia in a ram. The scrotal circumference of 22 cm was well below the 30 to 35 cm expected for a ram during the breeding season. (B) Unilateral testicular hypoplasia in a bull.

Because of the probable inherited basis of testicular hypoplasia, attempting to breed from an affected animal should be avoided and affected livestock males should be culled. Attempts at treatment with exogenous hormones are invariably unsuccessful.

Cryptorchidism

Perturbation of the normal process of testicular descent, such that one or both testes fail to complete their descent into the scrotum, results in the condition of cryptorchidism. Spermatogenesis is generally markedly impaired or absent in testes that are not scrotal due to severe disturbance in testicular thermoregulation. Animals that have a single cryptorchid testis are usually fertile, and there is often a compensatory increase in the size of the scrotal testis. When both testes are cryptorchid, the ejaculate is either aspermic or very severely oligospermic. Testosterone secretion is unaffected by cryptorchidism, so the libido of affected animals is normal. Indeed, it is far more common for cryptorchid animals to present as supposed castrates that exhibit masculine behaviour than it is to be a cause of subfertility in intact males. Cryptorchidism occurs most commonly in the stallion (Hayes 1986), boar (Huston et al. 1978) and some breeds of dog (Patterson 1977), but is rare in bulls, goats, and rams (e.g., Saunders & Ladds 1978, Janardhana et al. 1995, Greig 2000).

TABLE 36.1 Sites of testes in the cryptorchid stallion

Position of Left Testis	POSITION OF RIGHT TESTIS			
	Abdominal	Inguinal	Scrotal	All
Abdominal	125 (7.0%)	12 (0.7%)	545 (30.7%)	682
Inguinal	2 (0.001%)	69 (3.9%)	254 (14.3%)	325
Scrotal	291 (16.4%)	446 (25.1%)		
All	418	527		1744

The most common cause of cryptorchidism in ruminants is iatrogenic through unskilled use of rubber castration rings for castration. Misplacement of the ring can result in one testis being forced back either into the inguinal canal or, more commonly, into a subcutaneous position cranial to the scrotum. Occasionally, a high incidence of cryptorchidism has been recorded in individual flocks of sheep (e.g., Claxton & Yeates 1972). Such flocks, together with the clear breed incidences of the condition in other species (e.g., Red Danish cattle (Blom & Christensen 1947); Angora goat (Warwick 1961)), indicate the probability of an inherited basis for the disease. Indeed, Hobday (1903) recognised that the condition was probably inherited in the stallion. For this reason, the use of cryptorchid animals as sires should be avoided, and their castration is recommended.

In the dog, cryptorchidism is heritable and transmitted as a sex-limited autosomal recessive trait (Burke & Reynolds 1993). It is believed to be also heritable in the cat, possibly with a polygenic inheritance (Gradil & McCarthy 2013). In the dog the condition is found more commonly in smaller than larger breeds and in highly inbred lines; and cryptorchidism and other congenital defects of dogs are often found in association with each other. Retained testes exhibit a high incidence of neoplasia in both the dog and the stallion. The most common tumours of retained testes in dogs are seminomas and Sertoli cell tumours. In heavy-breed horses, many of these tumours are large teratomas. The risk of development of such tumours is a further reason for the castration of affected animals (Willis & Rudduck 1943, Pendergass & Hayes 1975).

Testes may be retained in the abdomen or inguinal canal. The incidence of different sites of retention in the stallion is shown in Table 36.1. In the dog, it is also more commonly unilateral than bilateral, with the right testis retained more often than the left (Cox et al. 1978). In supposedly castrated animals, diagnosis of the presence of a testis in the inguinal canal may be achieved by careful palpation of that region, but many cases require demonstration of the presence of male hormones for confirmation. All affected stallions exhibit testosterone concentrations that are not only higher than in castrated animals but also are responsive to LH stimulation (Cox et al. 1973, Cox 1975). After collection of an initial blood sample, 3000 IU of hCG is given intravenously, with a further blood sample collected 40 minutes later. Both are assayed for testosterone concentrations. In older animals (≥ 4 years), the presence of oestrone sulphate in the blood also confirms the presence of a retained testis. Similarly, in dogs, 2 µg/kg GnRH or 50 IU/kg hCG can be given and blood collected for testosterone concentrations about 1 hour later (doses in cats are cats 25 µg GnRH or 250 IU hCG) (Gradil & McCarthy 2013).

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Removal of retained testes from the may be effected by initial surgical exploration of the inguinal canal. Many testes will be found in this site, whereas others can be withdrawn from an abdominal position through the inguinal ring without much difficulty. Other abdominal testes can be withdrawn through a parapenile abdominal incision (Russell & Pollock 2011). Providing the gubernacular attachments of the testis are not disrupted during surgery, it is very uncommon to find an abdominal testis at a site other than near the internal inguinal ring. Gradil and McCarthy (2013) suggested that, in the dog, bilateral retained testes are often located caudal to the kidney, whereas a unilateral retained testis is more commonly located in the caudal abdomen lateral to the bladder. A unilateral retained testis should always be removed before the descended testis is removed or the animal may be falsely described as having been castrated.

Testicular Neoplasia

Testicular neoplasia is rare in the bull, ram, and boar, and, although common in dogs, rarely presents as a cause of infertility. Interstitial cell tumours are the most common tumour of the dog and are recorded occasionally as incidental findings in aged bulls; they are very rare in stallions. Seminoma, the next most common canine testicular tumour, are also occasionally found in bulls (and stallions), whereas Sertoli cell tumours rarely occur in species other than the dog. In cryptorchid stallions, a further testicular tumour is found relatively commonly: the teratoma, a benign growth that contains many different tissue types, including hair, bone, teeth, and cartilage (Fig. 36.34). Overall, testicular tumours account for over 10% of tumours in male dogs, with a considerably increased incidence in animals with cryptorchid testes (Taylor 2010).

Interstitial cell tumours usually occur in scrotal testes of aged dogs but are usually too small to be palpated. They may result in increased circulating concentrations of androgen and thereby predispose to androgen-related disease such as prostatic hyperplasia and circumanal gland adenoma. In bulls, interstitial cell tumours may cause irregular texture of the testis and, sometimes, enlargement. They cause no clinical signs in bulls and no impairment to fertility. This tumour is rare in the stallion.

The incidence of seminoma in cryptorchid dogs is about 20 times that of dogs with scrotal testes. Seminomas may become large but are generally innocuous in scrotal testes. They often grow slowly for long periods but may undergo a sudden increase in the rate of growth, for no apparent reason. The tumours may become necrotic or haemorrhagic, whereupon affected dogs may exhibit lameness, pain, crouching, or hunching. They occasionally metastasise to local lymph nodes. Seminomas are the most common equine testicular tumour.

Sertoli cell tumours are usually characterised by feminisation, in response to the tumour's oestrogen-secreting properties. Feminisation is typified by gynaecomastia, symmetrical alopecia, penile atrophy, a pendulous prepuce and attraction to male dogs, and occurs more often and to a greater extent if the neoplastic testis is inguinal or intra-abdominal than scrotal. If the tumour is unilateral, the contralateral testis is generally markedly atrophied. The oestrogenic secretion of the Sertoli cell tumour causes squamous metaplasia of the prostate gland, which may be of sufficient magnitude to cause obstructive uropathy. Metastases are uncommon but, when they occur, retain the oestrogen-secreting properties of the parent tumour. Sertoli cell tumours are rare in the station.

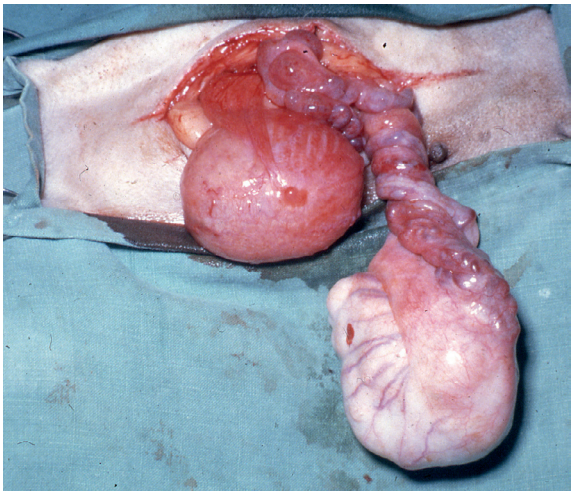
Teratoma (see Fig. 36.34) occurs most frequently in the stallion and very occasionally in other species. The tumour is most commonly found in cryptorchid testes, particularly of draught horses. They are either solid or cystic, and may have many tissue types identifiable within their substance. The tumours can become very large so that their removal may be quite difficult to achieve. However, they rarely metastasise.

In addition to their hormonal and malignant effects, tumours of undescended testes predispose to torsion of the spermatic cord, which results in gradual testicular infarction. In exceptional cases, both spermatic cords may be tightly twisted through several rotations (Fig. 36.35). Torsion of normal scrotal testes occurs occasionally (Young 1979), but the condition is most frequently associated with neoplastic changes in undescended gonads (Pearson & Kelly 1975). The susceptibility of such testes to tumour formation is therefore a strong justification for their removal.

Testicular Degeneration

The seminiferous epithelium of the testis is highly susceptible to external and internal insults that cause varying degrees of degeneration of this tissue. Testicular degeneration occurs in response to raised intratesticular temperature, toxins, endocrine disturbances, and infection (Humphrey & Ladds 1975, McEntee 1990). Some of the main causes of testicular degeneration in the bull are summarised

• **Fig. 36.34** Testicular teratoma in the undescended testis of a shire colt.



• **Fig. 36.35** Torsion of the spermatic cord of an undescended testis.

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TABLE 36.2 Causes of testicular degeneration in the bull

Temperature-Related	Environmental temperature Scrotal insulation (e.g., fat) Systemic pyrexia (but only if prolonged and high) Local inflammation, including contralateral orchitis Scrotal frostbite
Stress	Transport
Infectious Agents	Infectious bovine rhinotracheitis? Enteric viruses?
Dietary	Zinc deficiency Vitamin A deficiency?
Age-Related	Senile degeneration
Iatrogenic	Corticosteroid administration, dehorning before mating
Environmental	Radiation Heavy metals

From Parkinson & Bruere 2007.

• **Fig. 36.36** Scrotal mange of a ram. The inflammatory reaction that results in the crusty lesions of the scrotal skin raises intratesticular temperatures to a sufficient degree to result in substantial degeneration of the testis. (Courtesy Professor David West.)

in Table 36.2. Most causes of testicular degeneration do not manifest as infertility immediately as there is an interval of 4 to 8 weeks between the time at which the susceptible stage of spermatogenesis (particularly the primary spermatocyte) is affected and the time at which the sperm are finally ejaculated.

Causes

Temperature. Raised temperature in the testis can itself emanate from many causes. Many animals exhibit a period of subfertility during and after high summer temperatures. This phenomenon is well recognised in the boar (Althouse 2007), even in northern European conditions, and is sufficiently important in the bull for some AI studs to provide air conditioned accommodation for the sires to limit summer maxima of temperature (Roberts 1986). Temperature-induced summer subfertility of males has been recognised as a significant cause of poor reproductive performance of European breeds of domestic animal that are imported into tropical and subtropical regions. In rams, raised scrotal temperature can result from excessive amounts of wool over the scrotum or from leaving animals unshorn during the summer (Hulet et al. 1956). Thus showing rams in full fleece in midsummer can result in a significant proportion of such animals having reduced semen quality during the subsequent autumn breeding season. Deposition of fat in the neck of the scrotum, such as can occur in postpubertal bulls and rams fed high energy and protein diets, can prevent heat loss from the scrotum, resulting in subfertility and sometimes infertility (Jubb et al. 1993). Local inflammation to the scrotal skin or other structures in the scrotum can also raise the testicular temperature sufficiently to impair spermatogenesis (Rhodes 1976, Burke 1986, Roberts 1986). In the ram, scrotal mange, caused by *Chorioptes bovis* (Fig. 36.36), raises scrotal temperature sufficiently to cause testicular degeneration. Large numbers of rams can be affected in a flock, such that examination for the presence of the condition before the start of the breeding season is recommended (West et al. 2018). Trauma and abscessation of the scrotum (especially in rams after shearing) and facial eczema lesions on the scrotum also cause testicular degeneration, although inflammation of one testis or epididymis almost invariably causes a temperature-dependent degeneration in the opposite testis. Scrotal frostbite can cause testicular degeneration (Faulkner et al. 1967) due to the localised inflammation and necrosis.

Circulation. Abnormalities of the testicular circulation, such as occurs in varicocele, perturb the heat exchange mechanism responsible for maintaining the testis at a temperature lower than that of the body, again resulting in temperature-dependent testicular degeneration (Ott et al. 1982). This condition is rare in domestic species other than the sheep; however, in the Merino breed, it is sufficiently common to warrant routine examination of the spermatic cords during breeding soundness examination of rams (West et al. 2018). Degeneration results from inguinal or scrotal hernias for the same reason of disturbance in testicular thermoregulation. Pyrexia and often associated toxaemia caused by many infectious diseases can cause varying degrees of testicular degeneration.

Toxicities. A number of environmental and nutritional toxic causes of testicular degeneration have been recognised (Humphrey & Ladds 1975). Heavy metal or radiation contamination is a well-recognised cause of testicular degeneration, but many other materials have been implicated at various times. Over recent years, there has been increasing concern that environmental contamination with weakly oestrogenic substances has been adversely affecting human male fertility (Delbes et al. 2006). Effects of oestrogenic substances (e.g., clovers) on female fertility are well recognised (e.g., Shemesh & Shore 1994), but as yet there is little direct evidence of effects on the fertility of male domestic animals.

Stress. Stress-related degeneration occurs largely as a result of the inhibition of LH secretion by the corticosteroids that are released during stress (Welsh et al. 1981). Stress-related degeneration has been described in beef bulls after movement (Knudsen 1954, Jaskowski et al. 1961) and in other animals as a result of chronically unsuitable housing and management (Clarke & Tilbrook 1992). Hormonal degeneration is described in dogs whose gonadotrophin secretion is impaired either by a primary lesion of

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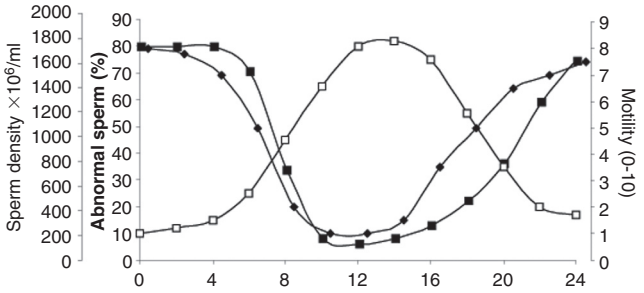


• **Fig. 36.37** Changes in semen quality of a bull during a period of testicular degeneration and the subsequent restoration of normal spermatogenesis. The cause of the degeneration was presumed to be high summer temperatures, density, motility, and proportion of abnormal sperm.

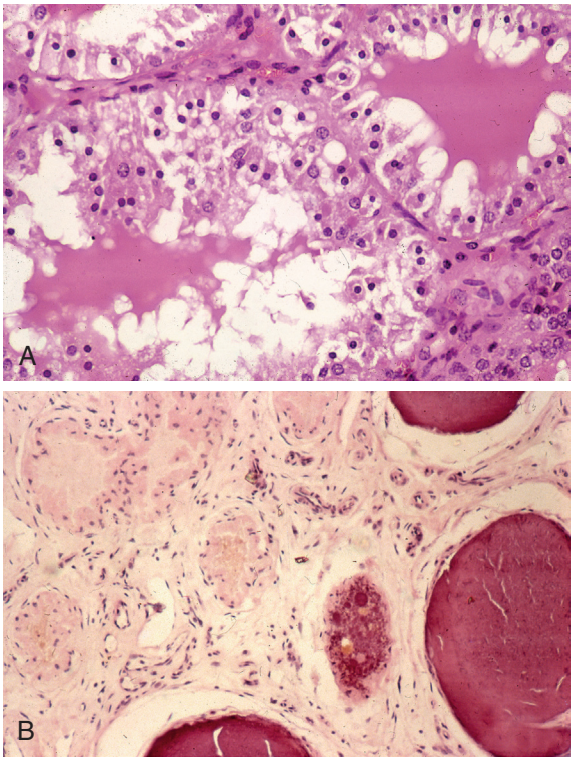
the anterior pituitary or because of the presence of oestrogenic tumours, such as Sertoli cell or adrenal tumours (Roberts 1986). Aged animals undergo a progressive, irreversible degeneration, initially characterised by increased percentages of sperm with primary abnormalities, with later oligospermia and testicular fibrosis (Bishop 1970, McEntee 1990).

Infections. Many infectious causes of degeneration have been described. The most severe of such infectious causes, orchitis, is considered under a separate heading, but many other, milder conditions affect the efficiency of spermatogenesis. Ascending infection by cytopathic bovine enteroviruses (BEV), infection with infectious bovine rhinotracheitis virus, bovine ephemeral fever virus, and, where it occurs, the epivag agent have all been specifically associated with testicular degeneration (Humphrey & Ladds 1975, Kirkland 2002). Bacterial contamination of such testes frequently leads an inherently mild bout of degeneration to progress to purulent or necrotic orchitis.

Depending on the severity and duration of insult, males may become moderate to severely subfertile or, in some cases, infertile. Libido is normally unaffected, as it is the seminiferous tubules rather than the Leydig cells that are affected by degeneration. The testes often feel softer and less resilient than normal, although they may still meet minimum thresholds for scrotal circumference. The tails of the epididymides are typically smaller and softer than normal. However, the most characteristic changes are in semen. Ejaculate volume is usually unaffected, but the number and motility of spermatozoa is reduced, and the proportion of sperm exhibiting abnormal morphology is significantly increased (Fig. 36.37). In severe cases, the ejaculate may become highly oligospermic with only morphologically abnormal sperm present. Abnormalities affecting the sperm head, midpiece, and tail may be present depending on when after the insult a sample of semen is examined.

The severity of the condition in terms of the extent to which semen quality is affected is poorly correlated with the prognosis for recovery. Some animals with severe semen changes can recover within a few weeks, whereas occasional animals with relatively mild changes never fully recover. When, or if, resolution occurs, it may be partial or complete. The extent of resolution depends on the degree of damage that is present in the seminiferous tubules. Provided that the stem cells (spermatogonia) and support cells (Sertoli cells) are undamaged and provided the lumen is not blocked with cellular debris, regeneration can take place. In more severe cases, permanent loss of seminiferous tubules occurs, with subsequent fibrosis and calcification (Fig. 36.38) of the testis. Such animals present with shrunken, firmer than normal, and very poorly resilient testes.

In the dog (Soderberg 1986) and stallion (Blanchard 2011, Threlfall 2011), testicular biopsy is potentially useful in determining



• **Fig. 36.38** Testicular degeneration in the bull. (A) Histology of the testis from bull with moderate testicular degeneration, showing loss of most meiotic and postmeiotic cells from the seminiferous epithelium. Healing may completely restore normal testicular architecture, or may result (B) in loss of seminiferous tubules, together with fibrosis and calcification of interstitial tissue. (With permission from Parkinson 1991.)

the prognosis for recovery. Intact basement membranes of the seminiferous tubules, the presence of spermatogonia within the tubules, and the patency of the lumina of the tubules all indicate a good prognosis for restoration of fertility (Kenney 1970). However, the technique should be used with great caution in these species because considerable damage to testicular parenchyma can result. In ruminants, biopsy is associated with a significant risk of haemorrhage and widespread tissue damage (Gassner & Hill 1955). Hence, it is best avoided or used with great caution. Trans-scrotal ultrasonography of the testes is potentially a much better alternative.

Hence, in practice, the only way that recovery can be demonstrated is through repeated semen examinations at 4- to 8-week intervals. Only when normal semen is present should the sire be cleared for service. An early sign of resolution, which occurs in some animals, is that the grossly abnormal morphology of the spermatozoa is replaced by relatively high percentages of cells with cytoplasmic droplets at the distal end of the midpiece.

Semen Changes During Testicular Degeneration

See Fig. 36.38. The initial changes in semen quality during testicular degeneration are a decrease in motility and an increase in the percentage of abnormal sperm, particularly sperm with proximal droplets and detached heads. If the semen is being cryopreserved, a precipitous decline in postthaw motility may occur at this stage. Subsequently, sperm numbers generally start to decline, although ejaculate volume is usually unaffected. As sperm numbers decrease, the proportion of morphologically abnormal sperm increases. These include abnormalities of the head, detached heads, and coiled

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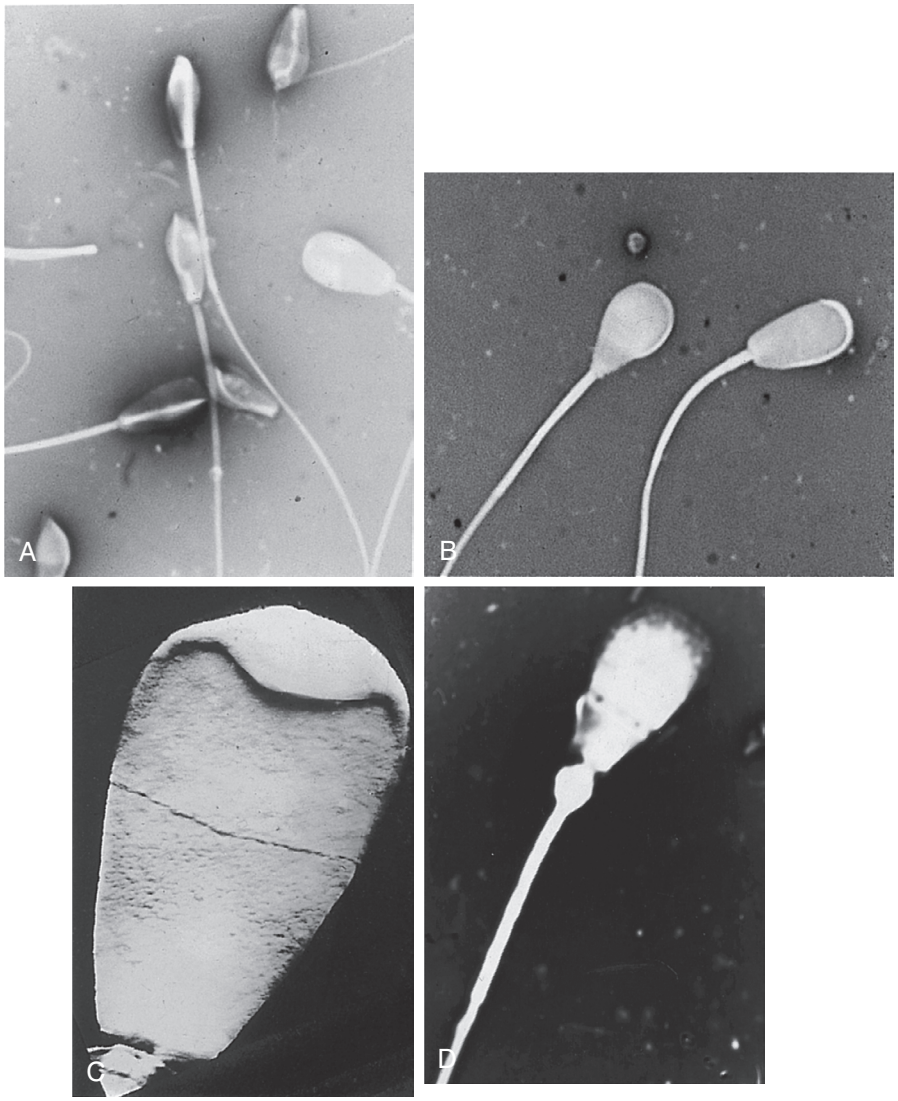

• **Fig. 36.39** Defects of the sperm head. (A) Abnormally shaped heads, usually associated with testicular degeneration. (B) Pyriform head contrasted with normal head. (C) 'Knobbed sperm' defect. (D) 'Diadem' defect.

tails. Bizarre abnormalities also occur in cases of severe testicular degeneration, including small abnormal heads, acrosomal defects, and the presence of premeiotic cells and stellate forms in the ejaculate. Sperm numbers may decline to the extent that the ejaculate becomes virtually aspermic.

During recovery, sperm morphology and motility tend to improve before sperm numbers. The percentage of sperm with distal droplets frequently increases during the recovery phase. Recovery may occur almost immediately after the nadir of semen quality but may be protracted. The extent and severity of semen changes detected in a sample at a single point in time cannot be correlated with either the duration of illness or the likelihood of recovery.

Sperm Abnormalities

Abnormalities of the Sperm Head

The sperm head consists of the genetic material, in the form of highly condensed chromatin, and key effectors of fertilisation (i.e., binding and passage through the zona pellucida) in the acrosome, inner acrosomal membrane and postequatorial zone. Most defects of the sperm head (Fig. 36.39A–D) arise as abnormalities of spermatogenesis and significantly impair fertility (Soderquist et al. 1991). It is common to find significantly raised percentages of these defects during testicular degeneration (see Fig. 36.35A), typically accompanied by a wide range of other sperm abnormalities. Abnormal condensation of chromatin (Johnson 1997) and abnormal nuclear shape (Ostermeier et al. 2001) are closely associated with reduced fertility.

Small changes in the overall size, acrosomal area, and width at the base of the head markedly reduce the ability of sperm to fertilise (reviewed by Barth & Oko 1989). The most common of such defects, the pyriform head, is readily recognisable by the narrow, elongated appearance of the head and the 'pinching' in of its postacrosomal region (see Fig. 36.39B). Nothling and Arndt (1995) reported that the pregnancy rate achieved by a bull with 36% pyriform heads was 46%, compared with 75% in a bull with normal semen. The abnormality impairs both fertilisation rate and subsequent failure of cleavage.

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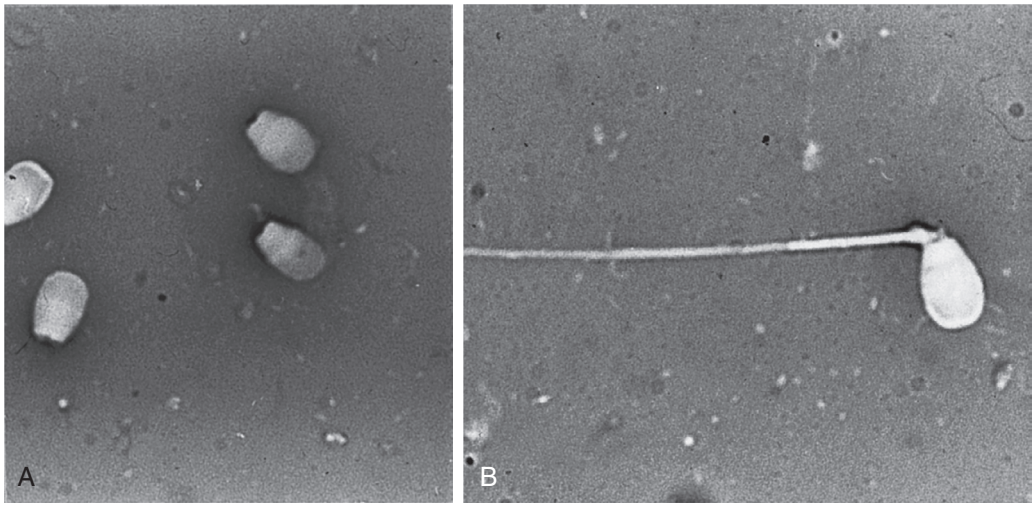

• **Fig. 36.40** Defects of the attachment of the sperm head. (A) Detached normal heads and (B) fractured neck.

Acrosomal Defects

Acrosomal defects are variably associated with reduced fertility. Many acrosomal defects arise as primary abnormalities of spermatogenesis, although acrosomal damage may also arise during epididymal transit and storage, or even after ejaculation. Many of the acrosomal defects that arise during spermatogenesis are present at high percentage in the ejaculate, in which case they are usually inherited, but identical abnormalities can be found at low percentages in most ejaculates, indicating that they can also arise spontaneously. Defects of the acrosome can be difficult to see in stained preparations, so the use of phase contrast or differential interference microscopy of wet mount preparations is recommended. The knobbed acrosome defect (see Fig. 36.39C) is the best known of the acrosomal defects. It is relatively easy to detect in well-made eosin–nigrosin (EN) smears. It is a common incidental finding at low percentages but can occur at high percentages (25%–100%) as a familial, probably inherited, defect. Moderate percentages of abnormal sperm are associated with reduced conception rates, whereas sires with high percentages are virtually sterile. Interestingly, in the bull, even though sperm with knobbed acrosomes are not themselves capable of fertilisation, *in vitro* fertilisation with apparently normal sperm from the same sires produced embryos with impaired development (Thundathil et al. 2000).

Detached and Swollen Acrosomes

Detached and swollen acrosomes may be observed in stained smears, although observation under phase contrast or differential interference contrast microscopy offers the best means of evaluating the acrosome. The site of origin of such defects is not always clear, for this abnormality may arise during spermatogenesis as a degenerative change in the sperm of a bull that has not ejaculated for some time. They may also occur as a tertiary defect in frozen-thawed semen of animals whose sperm do not survive cryopreservation well. Swollen acrosomes are difficult to interpret. At low percentages, detachment of the acrosome has been regarded as a minor defect, with fertility only being impaired with higher levels of the abnormality. However, recent data from the bull indicate that the percentage of detached acrosomes may be related in a linear fashion with fertility, and the significance of this abnormality is therefore being reassessed.

Other acrosomal abnormalities, including acrosomal crests and acrosomal folds, are variably associated with reduced fertility. For example, Meyer and Barth (2001) found that bulls with high percentages of abnormal acrosomes achieved normal conception rates except in competitive mating trials.

Nuclear Vacuoles

Nuclear vacuoles are abnormalities of spermatogenesis that arise either as a response to an insult to the testis or as congenital abnormalities. They are relatively difficult to visualise in EN smears but can readily be seen in wet preparations or with phase contrast microscopy. The best known is the diadem defect (see Fig. 36.39D), which can be seen as a series of refractile lesions at the base of the acrosome. Other sperm may have single or multiple apical vacuoles or large vacuoles that obviously distort the shape of the sperm head. It is common to observe occasional sperm with vacuoles in normal semen, and the abnormality can be temporarily present at high percentages for a short period after testicular degeneration (Barth & Oko 1989); however, bulls with high percentages (> 20%) of vacuolated sperm heads are considered subfertile.

Abnormalities of the Attachment of the Head, the Midpiece, and the Tail

Some abnormalities of attachment of the sperm head are inherited defects of the centriole or axoneme, occurring at high percentages, whereas others occur as acquired defects. Surprisingly, many such defects have minor effects upon fertility unless present at high percentages. Detached normal heads (Fig. 36.40A) occur commonly at low percentages in normal ejaculates, with higher percentages detected in first collections if males have not been serving or masturbating. Johnson (1997) showed that the presence of 30% to 40% of detached heads in an ejaculate was not associated with infertility. Detached heads (often accompanied by weak head–neck attachments and/or swollen acrosomes) can occur as degenerative changes in senescent sperm. The separated tails are immotile. Sperm with fractures of the attachment between head and tail (fractured neck; see Fig. 36.40B) may arise from senescent changes or due to congenital weakness of the attachment.

Two conditions that seriously affect fertility need to be differentiated from simple detached normal heads. First, there is the

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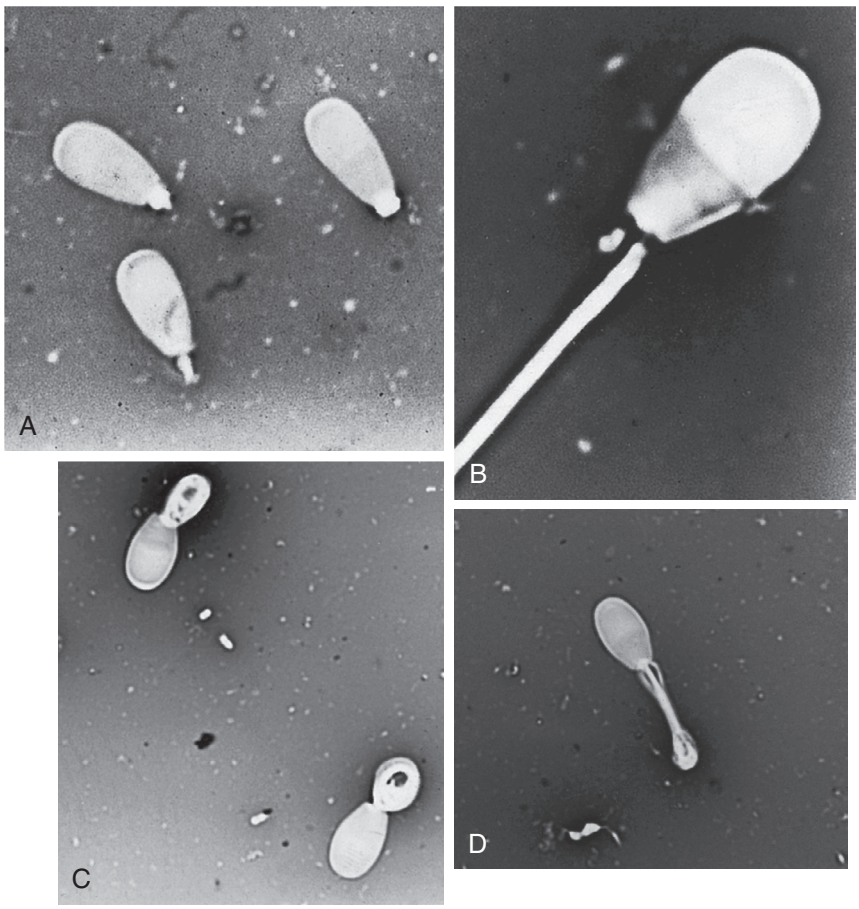

• **Fig. 36.41** Defects of the sperm midpiece. (A) 'Tail stump' defect, (B) accessory tail, (C) 'coiled tail', a defect of formation of the midpiece, and (D) the 'Dag' defect.

decapitated syndrome, which has been shown to be inherited in Guernsey and Hereford bulls (Blom & Birch-Anderson 1970, Blom 1977a), in which most sperm are decapitated, and the detached tails are motile. The semen of such bulls exhibits apparently normal wave motion. Secondly, the 'tail stump' defect occurs as an inherited condition of several breeds of bull (Blom & Birch-Anderson 1980), in which morphologically normal heads are attached to a vestigial structure that appears like a protoplasmic droplet (Fig. 36.41A). On electron microscopy, this droplet-like structure can be seen to consist of small segments of flagellar material and represents a vestigial tail. Affected bulls are sterile.

Abaxial implantation of the tail is generally of minor significance or should not be regarded as an abnormality (Barth 1989, Pant et al. 2002). Some degree of abaxial implantation may be regarded as normal in the stallion. Accessory tails, a curious, additional vestigial tail (see Fig. 36.41B) beside the main flagellum, can adversely affect fertility if the abnormality is present in a high percentage (e.g., Williams & Savage 1925).

Most other defects of development of the midpiece and tail arise as defects of spermatogenesis. Sperm with these abnormalities are either immotile or have subnormal motility and are associated with impaired fertility. Sperm with gross deformities of the tail and midpiece are often found in association with a wide range of other abnormalities (i.e., abnormal heads, detached heads, proximal droplets, etc.) in animals that are suffering from testicular degeneration (Parkinson & Bruere 2007). The coiled tail (see Fig. 36.41C) is commonly found during testicular degeneration. In the somewhat similar Dag defect (Blom 1966; see Fig. 36.41D), the apparently loose coils of the sperm tail represent a serious perturbation of the genesis of the flagellum, resulting in an immotile sperm. The defect occurs in variable percentages as a temporary acquired lesion, typically as one of the changes associated with testicular degeneration. When the condition is permanent, it is characteristically present in high (50%–100%) percentages, and in such animals, it is likely to be inherited. It is a common inherited defect of the Jersey breed.

Other, less spectacular, but nevertheless serious, defects of the midpiece occur. It is common to find sperm with an abnormally thickened midpiece, particularly at the point of attachment to the sperm head. This abnormality most often appears in conjunction with other abnormalities as a temporary response to some testicular insult. The corkscrew defect (so-called because the loose arrangement of the helix of mitochondria gives the appearance of a corkscrew to the midpiece of the sperm) is likely to be an inherited defect when present at high percentages.

Other abnormalities of the midpiece and tail include terminally coiled tails (Fig. 36.42A) and the distal midpiece reflex abnormality, which resembles a looped tail with a retained distal droplet enclosed within the loop (see Fig. 36.42B). Its significance for fertility is unclear, although it causes impaired fertility in natural service sires when present at a high (> 30%) percentage. The simple looped tail (i.e., with no enclosed droplet; see Fig. 36.42C) most commonly occurs as a postejaculation defect in response to poor temperature control of the semen, or hypotonic stress such as may

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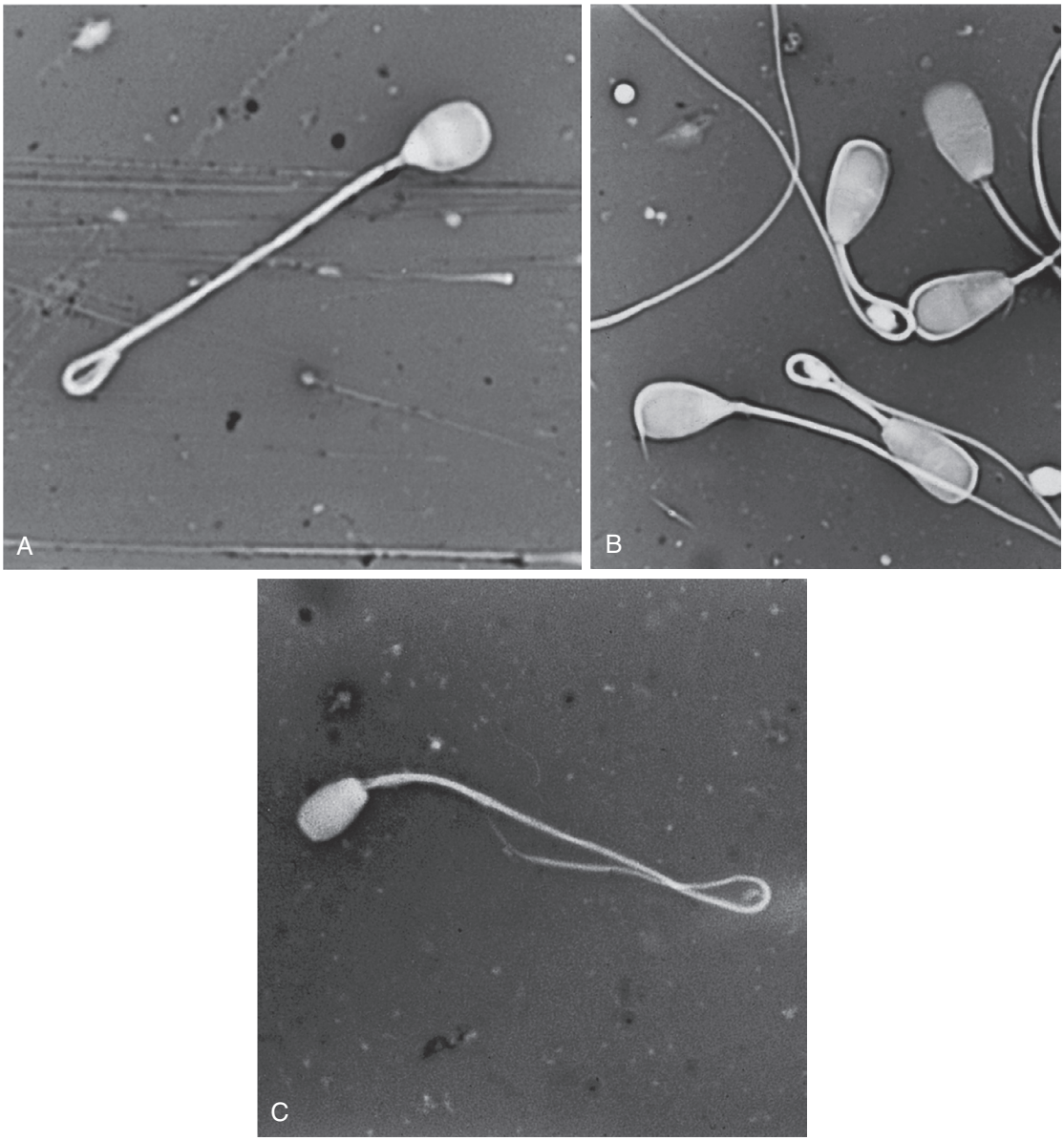

• **Fig. 36.42** Abnormalities of the sperm tail. (A) Terminally coiled tail, (B) distal midpiece reflex (appears as a looped tail with a cytoplasmic remnant enclosed in the loop), (C) looped tail.

occur if the semen becomes contaminated by water. Departure of seminal pH from its normal range can also cause looped tails, and they may therefore be an early indicator of the increase in pH that occurs during infection of the accessory glands. Looped tails can also be present with other abnormalities in animals experiencing testicular degeneration.

Cytoplasmic Droplets

The residual cytoplasm that remains at the end of spermiation is removed during the passage of sperm through the epididymis as a maturational change. The presence of sperm with protoplasmic droplets therefore indicates that epididymal maturation is incomplete by the time of ejaculation. Sperm with droplets close to the head (proximal droplets; Fig. 36.43A) are more immature than those with droplets at the distal end of the midpiece (distal droplets; see Fig. 36.43B).

Distal cytoplasmic droplets are not generally regarded as very significant abnormalities. High percentages of distal droplets (usually with a moderate percentage of proximal droplets) are commonly present in young bulls that are still peripubertal. The proportion of sperm with these abnormalities should decline to normal levels shortly thereafter (Evans et al. 1995, Johnson et al. 1998, Arteaga et al. 2001, Padrik & Jaakma 2002).

Proximal cytoplasmic droplets are more serious abnormalities, which result from epididymal dysfunction or (especially if present with mixed abnormalities) are a consequence of impaired spermatogenesis. When present at any more than low percentages, proximal droplets are associated with significant impairment of fertility (Soderquist et al. 1991, Saacke et al. 1995). For example, Blom (1977b), among others, suggested that ejaculates containing as little as 5% to 10% of proximal droplets are associated with reduced fertility. Furthermore, when sperm with proximal

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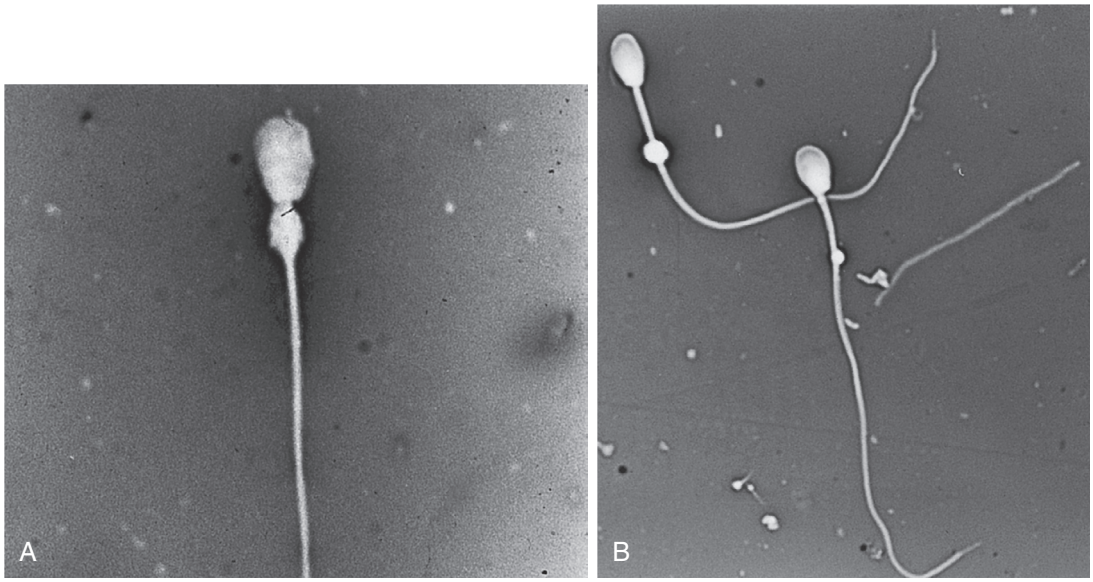

• **Fig. 36.43** Cytoplasmic droplets. (A) Proximal droplet and (B) distal droplet.

droplets are used in IVF, cleavage rates of embryos are poor (Amann et al. 2000).

Protoplasmic droplets are often present in the ejaculates of young sires that are being overused (Barth 1997). In such animals, daily sperm production rates are lower than in mature animals; in addition, the epididymis has not fully developed to its final length. Thus, if young sires are heavily used, as in AI centres, careful monitoring of the sperm morphology is strongly recommended. The majority of older bulls have only low but repeatable percentages of proximal droplets (Soderquist et al. 1996), but the mean percentage of affected sperm may increase during warmer seasons of the year. When increased proximal droplets occur in mature bulls (often with other abnormalities), it signifies a disturbance of spermatogenesis.

Lesions of the Epididymis and Mesonephric Ducts

Epididymitis

Epididymitis can also occur as a primary infection or by spread from an infected testis (Humphrey & Ladds 1975). Orchitis in the associated testis can also occur after a primary epididymitis. The general signs of epididymitis are similar to those of orchitis, namely heat, swelling, and pain of the affected part of the epididymis. Marked inflammation of the epididymis causes obstruction of the single, highly convoluted tube of which the organ is composed, so a loss of function normally ensues. Unilateral epididymitis typically results in some degree of subfertility, whereas bilateral obstruction can result in sterility. Furthermore, as with orchitis, unilateral epididymitis causes temperature-induced degeneration in the contralateral testis, so early antibiotic and nonsteroidal antiinflammatory therapy and cold hosing of the scrotum, or, in severe cases, orchidectomy is recommended.

Brucella ovis

In Australasia, North and South America, and central Europe, *Brucella ovis* causes epididymitis that results in epididymal obstruction and granuloma formation (West et al. 2018). It is a significant cause of infertility in rams in these countries. In an Australian (Queensland) survey, epididymitis was present in 58% of culled rams (Foster et al. 1989). Even when *B. ovis* infection is not associated with clinical evidence of epididymitis, its presence in the semen of rams is associated with impaired semen quality (Kott et al. 1988). The organism is transmitted between rams by homosexual behaviour (Jebson et al. 1954) and may be transmitted between ewes and rams as a true venereal disease (Hartley et al. 1955). It also affects stags and can be transmitted from rams to stags by direct (rather than environmental) contact (West et al. 1999, Riddler et al. 2000). Control of *B. ovis* is by identification and elimination of infected rams (Riddler 2002). False-negative serological tests (Robles et al. 1998) mean that repeated sampling and/or culture is needed to identify all infected rams. A voluntary eradication scheme in New Zealand and Australia has been successful in eliminating the infection from a large number of flocks (Riddler 2002).

Pleomorphic Gram-Negative Organisms

Ovine epididymitis also occurs as a consequence of infection with *Actinobacillus seminis* and *Histophilus ovis* (Walker et al. 1988, West 2004). Infection with these organisms is common in virgin rams and ram-lamb-producing flocks (Bagley et al. 1985), typically affecting rams in the peripubertal period. When the organism can be recovered from a high proportion of rams (Walker & LeaMaster 1986). Fortunately, only a relatively low proportion of animals progress to develop epididymitis. The possibility of venereal transmission has been suggested, and infection has been established by experimental intrapreputial and intraurethral inoculation (Al-Katib & Dennis 2005). Furthermore, these organisms undoubtedly infect ewes, causing subfertility (Lees et al. 1990), abortion (McDowell et al. 1994, Foster et al. 1999), and, at least experimentally, mastitis (Alsenosy & Dennis 1985). The infection is found in semen donor rams (Low et al. 1995), and anecdotal evidence exists for transmission between teaser ewes and semen donors. The measures for controlling the disease are poorly understood. Avoiding holding ram lambs in yards for prolonged periods and reducing pasture stocking densities have been suggested (West et al. 2018); however, culling animals with clinical epididymitis reduces the

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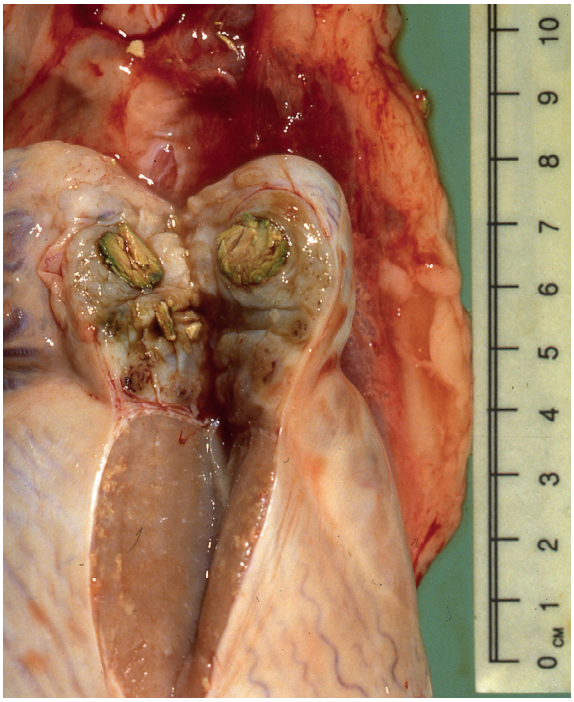


• **Fig. 36.44** *Actinobacillus seminis* infection of the head of epididymis of a 4-year-old Suffolk ram. Inspissated material was present in several large, abscess-like lesions within the head of both epididymides.

incidence but does not eliminate the disease (Bagley et al. 1985). Lesions are most common in the tail of the epididymis of both yearling and mature rams, where they are often associated with spermatoceles (Walker et al. 1986). Lesions also commonly occur in the head of the epididymis (Fig. 36.44). Recent development of PCR methods is likely to improve the identification of infected and carrier animals (Saunders et al. 2007).

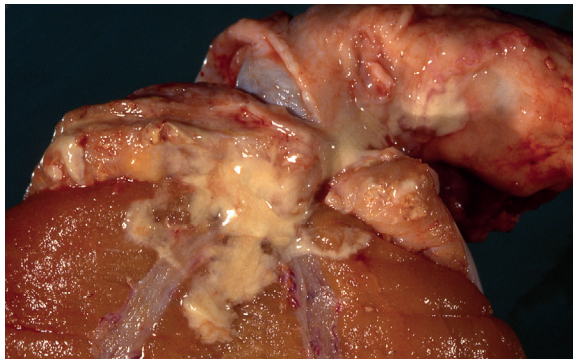
Histophilus somni infections have also been associated with orchitis (Corbel et al. 1986, Plagemann & Mutters 1991) and infertility in cattle.

Sperm Granuloma

Obstruction of the epididymis can occur after localised rupture (secondary to trauma or epididymitis) of the duct and leakage of sperm into the surrounding stromal tissue. The granuloma formed in response to the presence of foreign (sperm) antigen in the tissue cause an obstruction of the epididymides (Parkinson et al. 1993; Fig. 36.45). Leakage of sperm into the tail of the epididymis also occurs in animals that have been vasectomised. In vasectomised rams, the epididymal tail can be nearly as large as the testis (Fig. 36.46), where innumerable small sperm granulomata have formed. Surprisingly, this condition does not appear to be painful, and the libido of such vasectomised rams is normal.

Aplasia of the Mesonephric Ducts

Segmental aplasia of the mesonephric ducts (Blom & Christensen 1951) is most commonly manifested as an absence of parts of the epididymis (Fig. 36.47). In the bull, the condition is probably inherited (Konig et al. 1972). Absence of the head or tail of the epididymis can be determined relatively easily by careful palpation, but the medially sited epididymal body is often difficult to palpate. Epididymal aplasia also occurs sporadically in other species (McEntee 1990). There may be little or no effect on fertility



• **Fig. 36.45** Abscess-like sperm granuloma formation in the head of the epididymis of a Devon bull after rupture of the epididymal duct. (With permission from Parkinson et al. 1993.)

• **Fig. 36.46** Multiple sperm granulomas in the tail of the epididymis of a vasectomised ram.

• **Fig. 36.47** Aplasia of the mesonephric ducts in the bull. Absence of the entire left epididymis.

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of naturally mated animals as often there is some compensatory increase in the size of the unaffected testes.

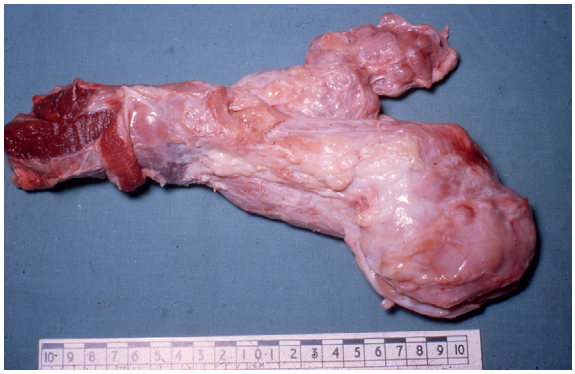
Aplasia of the ductus deferens is less common. In bulls (Blom & Christensen 1951) the condition may sometimes be diagnosed by rectal palpation, when strictures or dilations distal to the site of stricture may be discerned.

Lesions of the Accessory Glands

Vesicular Glands

Infection of the vesicular glands (*seminal vesiculitis, vesicular adenitis*) is relatively common in bulls. The incidence ranges between 0.2% (Blom 1979) and 9% (Bagshaw & Ladds 1974), and, in a more recent study (Scicchitano et al. 2006), a prevalence of 0.4% was reported. *Trueperella pyogenes* is considered the major cause of the destructive form of seminal vesiculitis (pathological changes readily detected by rectal palpation); however, a wide variety of bacteria, including *Corynebacterium renale, Actinobacillus actinoides, E. coli, Pseudomonas aeruginosa,* streptococci, and staphylococci, have been cultured from affected glands. These latter organisms are commonly regarded as causing secondary infections of a previously damaged organ. Other primary causative organisms include *B. abortus, Histophilus somni,* and *Chlamydophila* spp., as well as epivag, enteroviruses and BoHV-1. *Mycoplasma bovigenitalium* (Al-Aubaidi et al. 1972), *M. bovis* (LaFaunce & McEntee 1982), and *Ureaplasma diversum* have also been implicated as primary causes of vesiculitis. Most of these cause the degenerative form of seminal vesiculitis. Seminal vesiculitis occurs most commonly in young bulls, 1 to 3 years of age. Several routes of infection are possible including haematogenous (secondary to rumenitis or systemic infection) and extension from orchitis or epididymitis. The disease is rare in rams.

Infection is more commonly unilateral but may be bilateral. Diagnosis of the condition can be often confirmed by rectal palpation of the vesicular glands (Fig. 36.48) which, in the case of *T. pyogenes* infection, are characteristically enlarged, tense, and painful during the acute phase or lobular and fibrous in the chronic phase. In the degenerative form of seminal vesiculitis, only some peripheral thickening of the gland may be detected on rectal palpation, and in some cases, no palpable changes are detected. It is not uncommon for abscesses to form in *T. pyogenes* infected glands that can burst, causing generalised peritonitis, or fistulate either into the rectum or ischiorectal fossa. The main consequence of infection of the vesicular gland is increased polymorphonuclear leucocytes in the semen which, in the case of *T. pyogenes* infection, may present as frank pus in the ejaculate, or brownish discolouration of the semen due to degeneration of red blood cells associated with inflammation of the gland. The effect on semen quality is variable; in some cases there is decreased percentage of motile sperm and increased percentage of abnormal sperm, and in other cases there is apparently no effect on quality of sperm or fertility. Occasionally, during the acute phase of the disease, localised peritonitis may occur in the caudal abdomen, causing the bull to be reluctant to serve. Although bulls with seminal vesiculitis pose a potential risk for venereal transmission of the causative microorganism, there are few documented cases of this occurring. Cases of venereal transmission have been reported in bulls infected with streptococci (e.g., Webster 1932), with associated herd infertility, a yellowish-white postcoital discharge, and pronounced cervicitis.



• **Fig. 36.48** Seminal vesiculitis (vesicular adenitis) in the bull, showing asymmetrical enlargement of the vesicular glands.

Antimicrobial treatment is commonly attempted as the initial treatment. New, long-acting macrolides (e.g., tilmicosin, tulathromycin) have significantly improved the logistics and cost effectiveness of such treatment. It should be noted that in bulls of 9 to 12 months of age, there is a substantial spontaneous recovery rate and no benefit from early versus late start of treatment (Rovay et al. 2008), so it is probably worthwhile just waiting with such cases to see if they improve. Alternatively, direct injection of antibiotic into the affected gland has been described as being efficacious in a proportion of cases that fail to respond to parenteral antibiotics; Martinez et al. (2008) described recovery of yearling beef bulls within 3 weeks after intraglandular injection of ceftiofur and/or penicillin. Ablation of a unilaterally infected gland by intraglandular injection of 4% formaldehyde solution has been described as being moderately successful in restoring fertility to affected bulls (Waguespack et al. 2011). Alternatively, an infected vesicular gland can be surgically amputated, a procedure that is sometimes used for valuable AI sires (see Gilbert & Fubini 2004, for a description of the method).

Infection of the vesicular glands also occurs in the stallion (Blanchard et al. 1988, Varner & Schumacher 2011), from which many different organisms have been isolated (*B. abortus, Klebsiella pneumoniae, P. aeruginosa,* streptococci, and staphylococci). It may be one of the differential diagnoses of colic in the stallion (Freestone et al. 1993). Transrectal ultrasonography is a useful adjunct or, indeed, a substitute for rectal palpation in the diagnosis of such cases. Treatment may be attempted with antibiotics (Freestone et al. 1993), although such therapy is by no means always successful (Blanchard et al. 1988), possibly depending on the pharmakinetics of the antibiotic selected. Love (2007) suggested that direct administration of antibiotics into the infected gland via an endoscope is more effective than parenteral treatment.

Prostate

Prostatic disease is common in the dog (Johnston et al. 2001) but exceedingly rare in other species. Diseases of the canine prostate have been classified as: benign hyperplasia/hypertrophy; benign haemorrhage; squamous metaplasia; prostatitis (acute or chronic or resulting in abscess formation); prostatic cysts; and neoplasia (Williams & Niles 1999, Johnston et al. 2001). Many of these disorders are androgen-dependent and accompany the progressive enlargement of the prostate that is a normal part of the aging process of male dogs. When clinical signs occur, they are relatively similar regardless of the underlying pathology. Signs include urinary tract infections or dysuria, abnormalities of defecation, and haematuria or urethral bleeding. Systemic illness, alterations in gait, and infertility are less common signs (Krawiec & Heflin 1992, Williams & Niles 1999). Diagnosis is initially by digital rectal palpation, with confirmation when required by ultrasonography or radiography, biopsy or cytology (including collection of fine

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needle aspirates), and urinalysis (presence of polymorphonuclear leucocytes, bacteria, and blood). Prostatitis is probably the most significant of the disorders of the canine prostate that interfere with fertility. The route of infection is generally ascending, with a wide range of bacteria (such as *E. coli, Proteus* spp., staphylococci, streptococci, enterococci, *Pseudomonas* spp.) and mycoplasmas commonly being cultured. Prostatitis and prostatic hyperplasia often occur together, the prostate undergoing a diffuse or local suppurative reaction with a tendency to abscess formation. Prostatitis is treated with broad spectrum antibiotics, whereas hyperplasia, being androgen-dependent, is best treated by the administration of progestogens or by castration. Oestrogens, which were long considered to be of value in the treatment of prostatic hyperplasia, are now regarded as being contraindicated (Williams & Niles 1999). For males intended for use in breeding, medical therapy with the antiandrogen drug finasteride has been shown to be effective (Krekeler 2010). Finasteride blocks the action of 5α–reductase, the enzyme that converts testosterone to dihydrotestosterone (i.e., the hormone that causes prostatic hyperplasia).

Ampullae

A relatively common disorder of the stallion is partial or complete blockage of the ampullae with sperm (Love 2007). The condition typically occurs at the start of the breeding season or after a period of sexual inactivity and presents with oligospermia or ejaculation of semen containing a high proportion of immotile sperm and/or sperm with detached heads. Treatment is by ampullary massage, parenteral oxytocin, or maintaining a very high frequency of ejaculation.

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PART 6

Reproduction, Breeding, Reproductive Disease and Infertility in Less Common Domestic Species

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Old and New World Camelids

GAYLE D. HALLOWELL

South American Camelidae

There are four species of New World camelids: *Lama glama* (llama), *Lama pacos* (alpaca), *Lama guanacoe* (guanaco), and *Vicugna vicugna* (Vicuna). They originated in North America and have the greatest numbers in South America but are now reared worldwide as companion animals and for high quality fibre production. Outside of South America, they are reared much less for their meat. Adult llamas weigh 130 to 160 kg and can reach 1.9 m in height. Alpacas weigh between 40 and 65 kg and stand up to 1.5 m in height. There are two phenotypes of alpaca – Huacaya (Fig. 37.1) and Suri; they are different in appearance and the latter is less well adapted at very high altitudes (> 4200m; Fernandez-Saga 1975). The Huacaya alpacas have a shorter, denser coat and shorter nose than the Suri. Guanaco and llamas are a similar weight and size. Vicunas are the smallest of the four species; they weigh between 30 and 50 kg and stand a maximum of 1.3 m in height. They are primarily found in Peru and are a protected species. Normally, a healthy female can produce a cria every year, with 20 crias produced in her lifetime. All four species can interbreed and produce fertile hybrids. Hybridisation between a male Arabian camel and a female guanaco has also been achieved (Skidmore et al. 2001). There are still many unknowns regarding camelid reproduction compared with other species, but this chapter will aim to present current understanding, which will primarily be based on what is known in llamas, alpacas, and then camels.

Normal Reproductive Anatomy

Female

The ovaries of the llama and alpaca are ellipsoid to globular. Their size is dependent on the structures on the ovary being approximately 1 to 2 cm in diameter. In adults, multiple follicles of 2 to 5 mm are found on the ovarian surface (Fowler et al. 1998). These can be identified using transrectal ultrasound up to 3 mm in diameter; larger follicles and corpora lutea can be palpated *per rectum*. They have long, tortuous, firm uterine tubes (1–2 cm), which lie within the mesosalpinx. The uterus is bicornuate, which resembles a letter 'Y'. The body is short (3–6 cm) and horns are long (20–25 cm), with the left horn being slightly larger than the right, even in the nulliparous female. The nongravid uterus lies within the pelvic inlet. The uterus and cervix appear heterogenous during follicular dominance, whereas cervical folds become prominent and increase in echogenicity during the luteal phase. The uterine horns become straighter and have increased tone with increased oestrogen concentrations, whereas they are curled with minimal tone with increased progesterone concentrations. The cervix is 2 to 5 cm long and 2 to 4 cm in diameter, with two to three spiral-shaped cervical rings. The vagina is 10 to 15 cm long in the alpaca and 15 to 25 cm in the llama with a diameter of 2 to 5 cm. The vulva is small and does not change in appearance with changes in hormones or pregnancy.

Male

The scrotum is nonpendulous and is situated high in the perineal region and level with the ischial arch. The testes are relatively small compared with ruminants of comparable body size, and the epididymis lies on the dorsal border of the testis with the head situated at the cranial pole. The testes develop slowly. They are present in the scrotum at birth but are hard to palpate; at 6 months, they are small and soft, but by 12 months, they can be easily palpated and the testis and epididymis easily differentiated. It is at around 12 months of age that future herd sires can be selected based on testis size and other physical characteristics. The prepuce, which is flattened and triangular in shape, lies in the perineal region. The glans penis and prepuce are attached until the male reaches 2 to 3 years of age, such that the penis cannot be exteriorised in young males. When animals are not sexually aroused, the preputial orifice faces caudally. When sexually aroused, the developed preputial musculature allows for redirection of the orifice cranially. The fibroelastic penis is 35 to 45 cm in length and is retracted by the presence of a prescrotal sigmoid flexure. The glans penis is 10 to 12 cm in length, and the distal tip consists of a cartilaginous process. The urethral orifice is at the base of the cartilaginous process and not at the tip (Tibary & Vaughan 2006). The curved nature of the cartilaginous process enables penetration of the cervix through rotational and thrusting movements, allowing uterine deposition of semen. A dorsal urethral diverticulum prevents insertion of a catheter along the urethra into the bladder. Camelids have no seminal vesicles but have well developed bulbourethral glands and ampullae of the vas deferens.

Safe Restraint for Reproductive Examination

The majority of llamas and alpacas are relatively easy to handle. In many animals a calm, quiet approach will allow it to be done without sedation. Halters, a stable area, a wall to prevent the animal moving away from the examiner and specific crushes, if available, can allow a reproductive examination to be performed safely and effectively. A small number of animals, usually males, may require sedation using opioids or alpha-2 agonists combined

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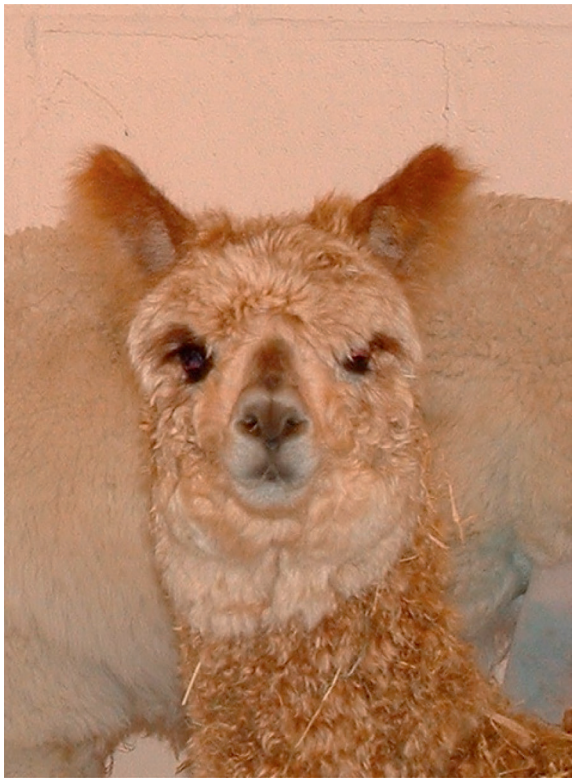

• **Fig. 37.1** Characteristic appearance of a Huacaya alpaca.

with opioids. Usually, adverse responses to handling include spitting, kicking, and biting.

Puberty, Sexual Maturity, and the Breeding Season

The precise onset of puberty in llamas and alpacas is unknown, but nutritional status and environmental conditions are thought to play a key role. Increases in ovarian hormone production have been recorded in well-developed llamas at 5 months, and ovarian activity detected in alpacas at 10 months. A relationship exists between successful mating and body weight with successful mating occurring in alpacas at 12 to 14 months at a body weight of 40 kg. It is recommended that llamas and alpacas are first mated at 2 years of age or at two-thirds of their final body weight to prevent stunting of the dam and dystocia.

First production of spermatozoa in ejaculate has not been studied in this group, but puberty is thought to occur between 18 months and 2 years of age. Domesticated individuals are not used for breeding until 3 years of age (Bravo 2014).

Whether these species as a group are seasonal breeders is controversial. It appears to be more closely related to environmental conditions or management strategies rather than seasons per se. In their harsh natural habitat, they are seasonal breeders, in that they display sexual activity and give birth in the warm, rainy season. However, when reared in milder climates, they mate and give birth all year round. Thus they are nonseasonal breeders but can become so if required by environmental and nutritional factors.

Ovarian Activity, Mating, and Ovulation

These species are induced ovulators, requiring mating to trigger the LH surge required for ovulation. Thus they do not have regular oestrous cycles. Ovarian activity is thus described regarding the follicular and luteal phase. It is well documented, using a variety of modalities, that there are repeated follicular waves that regress without ovulation in the absence of mating. In a follicular wave, which comprises a very large number of individual follicles, often too numerous to count, some reach 4 to 5 mm in diameter, of which one becomes the dominant follicle; the others then undergo atresia and regress. The number of follicles is inversely proportional to the size of the dominant follicle (Vaughan 2002). Before the final regression of the dominant follicle, a new follicular wave develops, resulting in the presence of simultaneously regressing and growing follicles; this is known as 'overlapping follicular waves'. These are reported to occur for about 30% of a female's lifespan. It is not possible to differentiate by transrectal palpation or ultrasonography between 5 and 6 mm follicles that are growing or regressing.

The follicular wave duration is variable within and between species of camelids, being 20 to 25 days in llamas and 12 to 22 days in alpacas (Aba 2014). In llamas, the follicular growth phase is approximately 9 days, the plateau phase is 8 days, and the regression phase 8 days. In alpacas, the plateau phase is between 2 and 8 days, and the regression phase is 3 to 8 days. The maximum preovulatory size occurs 6 to 8 days into the growth phase in llamas, with the maximum diameter of the dominant follicle being 12 mm in llamas and 8 to 12 mm in alpacas. Follicles develop equally between the two ovaries. Peak plasma oestradiol-17β concentrations correspond with the maximal preovulatory size and occur 12 and 8 days from the beginning of the growth phase in llamas and alpacas, respectively. With overlapping follicular waves, oestradiol-17β concentrations remain elevated, causing the long periods of receptivity to the male (> 3 weeks) with only short periods stages when the male is rejected. Without follicular waves, when a preovulatory follicle (> 7 mm) is present, females will be receptive for several days. Males are usually rejected when plasma oestrogen concentrations are low but can be accepted even if preovulatory follicles are absent. There is a lack of information on the mechanisms surrounding follicular growth, but it does appear to be associated with changes in FSH secretions as occurs in other species (see Chapter 1). Anovulatory follicles have been described in llamas and alpacas – they last longer and are larger. Cystic follicles are also identified as structures larger than 12 to 14 mm and persisting for 25 to 30 days. They occur more frequently in nonmated animals, and some individuals appear to be more prone. It is debatable whether cystic and haemorrhagic follicles are one and the same; they do not secrete oestrogen and do not usually affect ovarian dynamics.

The males pursue females when they are in oestrus. The flehmen response is exhibited when the male sniffs the perineum, urine, or faeces of females; this is followed by the male lifting his head and opening his mouth, sometimes shaking the head rapidly. They do not curl the upper lip as in other species. Some females 'cush', that is, assume sternal recumbency when approached by a male, whereas others do not 'cush' until the male mounts and places pressure over their hindlimbs. Other receptive females often sit close to mating pairs. During mating, the males vocalise (a guttural sound called 'orgling'), dilate their nostrils, flag their tails, and demonstrate slight tremors of ears and nostrils with excitement. The females, in contrast, are very passive, although they

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may move from sternal to lateral recumbency. Mating usually lasts 20 to 30 minutes, and ejaculation occurs throughout this period (dribble ejaculation). Females who are not receptive will spit, kick, scream, and run away but will occasionally 'cush' and submit to mating. Continuous contact between males and females inhibits the male's desire to mate, despite the presence of other receptive females. However, when males are introduced, most females will be mated in the first week (Bravo 2014).

Ovulation of one follicle occurs when a neural signal triggered by mating involving both mounting and intromission stimulates the secretion of GnRH and then LH. Ovulation is not triggered simply by mounting (Fernandez-Saga et al. 1970). Multiple matings do not improve ovulation rates. There appears to be an ovulation-inducing factor (OIF) in the seminal fluid that has a GnRH-like activity stimulating LH release, with a systemic rather than local effect (Paolicchi et al. 1999, Adams et al. 2005, Ratto et al. 2010). Injections of hCG, GnRH, and LH can also induce ovulation (Fernandez-Saga et al. 1970). Increased oestradiol 17-β concentrations are seen on the day of mating and for up to 24 hours, and then fall 2 to 3 days later. Ovulation occurs 24 to 48 hours after mating. Ovulation failure has been reported after 10% to 30% of natural matings (Bravo 1994), multiple ovulations reported in approximately 10% of natural matings (Vaughan & Tibary 2006), and spontaneous ovulations have a prevalence of 3% to 10% (Bravo & Sumar 1989, Sumar 1996), the mechanism of which is unknown (England et al. 1969). The size of the dominant follicle (> 7 mm) determines whether ovulation occurs (Bravo et al. 1991). If there is no ovulation but luteinisation of usually regressing follicles occurs, the luteinised follicles last for 5 days, and then a new dominant follicle develops in a further 5 to 7 days (Bravo et al. 1991). If there is no ovulation and no formation of a luteinised follicle, the female will remain receptive to the male.

Pregnancy Diagnosis

There are many important aspects of breeding management, but timely and accurate pregnancy diagnosis plays a fundamental role. Once a female has passed a breeding soundness examination, she should be teased until receptive to the male, when she can be bred. Females should be teased 7 to 12 days after mating. Those females that are not receptive at 14, 21, and 28 days should be examined using transrectal ultrasound. If the female is receptive to the male, then she should be mated again. If after three mating attempts the female is still receptive to the male, an infertility investigation should be performed. Pregnancy evaluation, using transrectal ultrasound, should be performed at 14, 25, and 35 days and between 45 and 60 days (Pearson et al. 2014a); further examinations should be performed beyond this date if the animal has a history of pregnancy loss. Blood progesterone concentrations greater than 1 ng/mL can be used for pregnancy diagnosis but do not provide information regarding twinning or subsequent early pregnancy failure loss; it is not as reliable as ultrasonography. Transrectal palpation in large llamas can readily be performed, but examination in smaller llamas and alpacas is dependent on the hand size of the examiner. Care is required as in the horse. However, transrectal ultrasonography can be performed without the need to insert a hand or arm into the rectum by using an adapted stiffened linear transducer with a moderate to high frequency (7.5–13 MHz), mounted on a thin piece of plastic pipe.

Plasma oestrone sulphate has two peaks during pregnancy but is most useful to measure in the last trimester (last 90 days; Bravo et al. 1996a, Aba et al. 1998). Relaxin concentrations can also be used for pregnancy diagnosis if measured after 85 days; site of production is unknown (Bravo et al. 1996a). After 85 days the uterus has extended beyond the pelvic brim so that transabdominal ultrasonography is most useful at this stage for pregnancy diagnosis and assessment of fetal viability. A lower frequency (5 MHz) microconvex or convex transducer will be optimal at this stage.

Pregnancy and Parturition

The pregnant animal does not need specific care, although it is advisable to keep males away from females in the last trimester, as placental oestrogen production can lead to inappropriate mating attempts, which can cause vaginal trauma and disruption of the cervical plug. Almost all successful pregnancies are carried in the left uterine horn. Maternal recognition of pregnancy is not fully understood, but what is known suggests it is complex and occurs over a short time period, making embryo loss likely in early pregnancy. The CL is necessary for the maintenance of pregnancy throughout gestation. Progesterone initially falls 2 weeks before parturition and plummets along with oestradiol-17β in the last 24 hours. Gestation length is traditionally reported as 335 to 360 days in llamas and alpacas but may be significantly more variable outside South America, ranging from 320 to 400 days. Factors likely to influence gestation length include breed, fetal gender, season of year, nutrition, and sire and dam effects; twinning is rare. The placenta is similar to the mare and sow (see Chapter 4) and is classified as being diffuse, epitheliochorial, and microcotyledonary (Aba 2014).

Parturition usually occurs in the early hours of the day (07:00–13:00), which compliments their natural habitat and behaviours, when ambient temperatures plummet in the evening and at night, and allows for predator avoidance. This is particularly important as the dam does not lick the neonate dry nor does she consume the placenta. There is often minimal to no warning of impending parturition. Relaxation of the sacrosciatic ligament begins 2 weeks before parturition, vulval oedema a few days before, and elongation of the vulva on the day of parturition. The udder develops in the 2 weeks before parturition, normally with mammary oedema extending to the xiphoid. Colostrum is produced from 4 to 6 days before parturition. Changes in electrolytes of mammary gland secretions are not a reliable indicator of impending parturition.

Parturition is usually swift. The first stage varies greatly in length from 3 to 48 hours and manifests as inappetence, restlessness, anxiety, increased frequency of urination and defecation, repeated standing and 'cushing' as well as separation from the herd. Second stage labour usually lasts less than 30 minutes but can be longer in primiparous females. Normally, the head, then one forelimb, and then the second are delivered. Most fetuses are expelled with the dam standing. Expulsion of the placenta usually occurs in less than 30 to 60 minutes but may take up to 4 hours. There is rarely rejection of young, even when the female has poor nutritional status and mild to moderate illness of the dam. Alpaca crias weigh 6 to 8 kg and llama crias 9 to 14 kg at birth. Birth weight increases with age of dam. Perinatal mortality is reported to be low (6%; Bravo 2009).

Females may be receptive to males 24 hours after birth but are not fertile, as uterine involution has not occurred, and ovulatory follicles have not had time to develop. It is thus recommended not to breed until 2 to 3 weeks after parturition (Bravo 1994). Fertility increases from 30% at Day 10 to 70% at Day 30 postpartum (Bravo 1995).

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Induction of Parturition

Induction of parturition can be undertaken due to fetal distress or ill health of the dam but is not an appropriate management procedure (Rodriguez et al. 2014). Before induction of parturition is attempted, the presence of uterine torsion, which is a common finding, should be ruled out (Rodriguez et al. 2014). To minimise the risk of perinatal asphyxia syndrome, induction should only be undertaken if gestation is longer than 330 days, and colostrum is being produced. Induction can be achieved (see Chapter 6) using prostaglandin F2α (dinoprost tromethamine) or its analogue cloprostenol; the latter is preferred as the former has been associated with causing respiratory distress in the dam (Rodriguez et al. 2014). Oxytocin and dexamethasone should not be used because they are ineffective, and dexamethasone can result in fetal death *in utero* (Bravo et al. 1996b).

Dystocia

This is relatively rare, with 2% to 5% of animals needing assistance. Dystocia should be suspected if first stage labour lasts more than 6 hours and with increasing levels of pain or discomfort, or if second stage labour does not progress normally. Any abnormal discharge from the vulva (haemorrhagic or purulent) requires further evaluation.

The most common maternal causes of dystocia are failure of cervical dilatation and uterine torsion, but uterine inertia and fetopelvic disproportion (small pelvis) have also been reported (Tibary et al. 2008). Hypocalcaemia can cause uterine inertia. Dystocia as a result of fetomaternal disproportion due to small dam size is avoidable if animals are not bred until they are 65% of adult body weight and height.

Dystocia of fetal origin is usually due to fetopelvic disproportion (large fetus) or abnormal fetal disposition (see Chapter 14). Older llamas and alpacas produce larger fetuses, perhaps making this more likely. Dystocia due to fetal developmental malformation is rare.

Uterine Torsion

This is a common cause of colic and occurs at 8 to 10 months gestation and at parturition. Clinical signs are variable and include general depression, excessive vocalisation, mild to severe signs of colic (circling, kicking at the abdomen, lying in lateral recumbency), diarrhoea, and anorexia. It can be corrected by rolling, or surgically after an exploratory laparotomy.

Failure of or Incomplete Cervical Dilatation

This has been linked to long-term progesterone therapy used to maintain pregnancy or abnormal fetal disposition during first stage labour. Topical PGE_1 (misoprostol) applied to the cervix can be used to aid delivery of a dead fetus. However, surgical removal of the fetus via a Caesarean is recommended if the fetus is alive.

Abnormal Presentation, Position, or Posture

Head and Neck Deviations

These are the most common cause of dystocia in camelids. Due to the very long neck, correction can be challenging. Epidural anaesthesia facilitates correction, particularly if combined with the use of a soft rope positioned in the mouth and behind the ears. If correction proves impossible, a congenital abnormality such as ankylosis should be considered, and delivery should be performed via a Caesarean section. In llamas, it is possible to perform fetotomy by removing the head and neck if the fetus is dead (see Chapters 14 and 15) with the dam heavily sedated.

Carpal Flexion

This is relatively easy to correct if the fetus is repulsed and the foot cupped and lifted over the pelvic brim.

Management After Dystocia

After dystocia, the dam is at increased risk of postpartum complications such as postpartum metabolic derangements, haemorrhage, severe metritis, peritonitis, and sepsis; these are more likely after removal of a dead fetus. Fluid therapy should be instituted in animals demonstrating signs of hypovolaemia or shock. Antimicrobial therapy may be required, ideally using an intravenous, bactericidal, and broad spectrum agent such as trimethoprim sulpha.

The neonate often requires resuscitation, with attention to the airway to ensure an unobstructed airway. Mouth-to-snout resuscitation may be undertaken, but occasionally endotracheal intubation may be required. There is evidence in other species that oxygen is not beneficial and may be harmful during resuscitation attempts. Cardiovascular compromise may require chest compressions and administration of cardiac drugs, particularly the use of intravenous epinephrine (adrenaline). Doxopram is contraindicated in dystocia cases; it is ineffective for secondary apnoea, and because it increases cerebral oxygen demand, it can further predispose to perinatal asphyxia syndrome.

Disorders and Diseases of Pregnancy

Abortion

Determining the cause of abortion can be challenging, and a specific diagnosis is only reached in 30% of cases. There are a variety of causes of abortion, and as in other species, they can be infectious and noninfectious. Of the infectious causes, bacterial infections are the most prevalent. *Leptospira spp, Chlamydophila spp, Campylobacter fetus,* and *Listeria monocytogenes* have all been identified in camelids, as has *E. coli, Streptococcus spp, Trueperella pyogenes,* and *Psuedomonas spp.* Viral infection can also play a role, the most important of which is bovine viral diarrhoea virus (BVDV); persistently infected animals can occur as in cattle. The methods of diagnosis are the same as in cattle (see Chapter 24). Other viruses linked to abortion include equine herpes virus, equine arteritis virus, and blue tongue virus. The most common protozoal cause of abortion in camelids is *Neospora caninum,* but *Toxoplasma gondii* is also a suspected cause (Pearson et al. 2014b).

Many of the noninfectious causes of abortion are similar to those in other species. Ascending placentitis is rare in camelids, due to the cartilaginous rings found in the cervix, which result in a tight cervical seal. Ketosis is also rare compared with small ruminants. Vaginal prolapse (see Chapter 10) commonly occurs in the last 2 months of gestation, particularly in older animals. The methods of treating and managing the disorder are similar to those used in other species. Because there may be a genetic predisposition resulting in reoccurrence, affected females should not be bred from again. Prolonged gestation is a common occurrence in camelids, although some of these cases may reflect and underestimate of the wide variation in gestation length that can occur. The most common reason for prolonged pregnancy is abnormal placentation resulting in intrauterine growth retardation (Pearson et al. 2014b). Other causes include fescue toxicosis, nutritional deficiencies (particularly energy deficiencies), fetal abnormalities, and harsh climatic conditions. The most appropriate course of action

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for these animals is to allow them to go to term once pregnancy has been established, assuming the fetus is alive and dam is well.

Postpartum Disorders

Similar postpartum complications are as in other species. Retained fetal membranes do occur and rarely result in any systemic effects. Systemic inflammatory response syndrome (SIRS) can be seen in those that have secondary peritonitis. Treatment is the same as for other species (Pearson et al. 2014c). Postpartum haemorrhage is rare, but when it occurs, it is usually the rupture of the vaginal branch of the internal pudendal artery. Rupture often occurs after internal manipulation to correct dystocia, especially after fetotomy in the correction of dystocia. Bleeding from the vulva or intermittent expulsion of blood requires further internal examination and, if necessary, can be treated by digital pressure on the vessel and supportive care. The risk of future haemorrhage in subsequent pregnancies is unknown (Tibary et al. 2008). Uterine tears are not as common. The cause of postpartum recumbency is multifactorial, and it most often occurs after dystocia, although it can occur after a normal birth; it more frequently occurs secondary to metritis, necrotic vaginitis, retained fetal membranes, hypocalcaemia, pelvic and other musculoskeletal injuries, postpartum haemorrhage, uterine tears, and peritonitis (Pearson et al. 2014c).

Infertility and Subfertility

Female

Infertility and subfertility is a major problem in camelids. Congenital and acquired disorders both play a role. The majority of the causes relate to repeat breeding and are due to early pregnancy failure, with fewer problems attributed to abnormal behaviour and abnormal external genitalia (Tibary 2007, Tibary 2010a).

Repeat breeders are those that have failed to become pregnant and thus return to oestrus after three breeding cycles after mating to a male of known normal fertility. Factors responsible can be categorised as either fertilisation failure or early embryonic death. Fertilisation failure is most likely due to poor breeding technique, failure of ovulation, failure of ejaculation, disease of the uterine tube and ovarian bursa preventing normal ovum transport, and poor semen quality. Early embryonic loss can be due to abnormal embryos, poor uterine environment due to infection or fibrosis, CL dysfunction and poor management of the mother due to stress, nutritional deficiencies, heat stressor, and intercurrent disease (Tibary 2010a).

Early pregnancy loss can only be assumed if the female has been positively identified as being pregnant using ultrasound, but identifying the cause can be very challenging. Potential causes of this include: CL dysfunction, in which obesity and thyroid dysfunction have been shown to play a role; genetic or environmental factors, such as heat stress, twinning, abnormal uterine environment, vitamin and mineral deficiencies (A, D, and selenium); and negative energy balance, especially in females that are producing large quantities of milk (in first 2–4 weeks postpartum).

Specific Causes of Infertility

Ovarian Disease

Ovarian disease is the second most common causes of reproductive failure (Tibary 2010b). Haemorrhagic follicles or cysts are a common finding, but their effect on fertility is not well understood. In some animals, these will luteinise and will therefore result in the female being nonreceptive to the male. However, unlike in cattle, in most cases, ovarian follicular activity is maintained (Pearson et al. 2014d). Diagnosis is by ultrasonographic examination, and similar treatments to those used to treat follicular cysts in cows have been used (see Chapter 22) without success. Ovarian hypoplasia or dysgenesis is a common finding in young animals and is diagnosed on repeated ultrasonographic examinations and laparoscopy. These animals, unlike in other species, display persistent receptivity to the male (Pearson et al. 2007).

Persistent luteal activity due to a persistent CL or luteinised haemorrhagic follicle with high blood progesterone concentrations (> 1.5 ng/mL) are permanently nonreceptive to the male. Diagnosis is relatively straightforward with ultrasonography and blood progesterone assays. Treatment with cloprostenol is recommended. Dinoprost tromethamine should be avoided due to reports of respiratory distress in some animals. Some individuals require two or three treatments, and these conditions can recur.

Uterotubal Disease

The most common disorder, which is still relatively rare, is salpingitis. The uterine tube is often fluid filled and can be identified ultrasonographically.

Uterine Disease

The most common uterine disorders reported are endometritis and endometrial fibrosis. Endometritis is the most common acquired disease resulting in infertility; little is known regarding its pathogenesis. Major contributing factors are thought to include too frequent breeding, postpartum complications that include dystocia and retained fetal membranes, inappropriate breeding practices (routine uterine lavage and antimicrobials postbreeding), selenium and copper deficiencies, and inappropriate nutrition resulting in obesity and emaciation. Diagnosis is made based on a history of repeat breeding or early embryonic death, the appearance of external genitalia where a thick mucopurulent discharge will be evident, and transrectal ultrasonography where the presence of intrauterine fluid and a thickened uterine wall will be evident. Further diagnostic tests, such as vaginoscopy, uterine cytology, swabbing and culture that include antibiotic sensitivity tests, and uterine biopsy in those animals that are barren, should be done. Many different Gram-positive and Gram-negative bacteria have been isolated as causative agents of endometritis. The approach to management of this condition is similar to the mare (Tibary 2010a).

Cervical Disease

The most common cervical abnormalities identified are acquired adhesions or tears, usually secondary to trauma at parturition or too frequent breeding. These can be identified on direct cervical examination with a speculum or with video endoscopy. This condition has a poor prognosis for future normal reproductive function.

Vaginal and Vulval Disease

The most common diseases identified are vulva hypoplasia and atresia and a persistent hymen. As these may be associated with a genetic predisposition, it is debatable whether these animals should be used for breeding. Vulvar atresia is usually noted in neonates, resulting in the accumulation of urine in the vagina, vestibule and uterus; urine can 'back up' into the bladder and even the kidneys (Hallowell et al. 2007). Persistent hymen is seen in young animals that are nonreceptive, permanently receptive, or that display pain during mating. It can also be associated with more serious congenital anomalies such as segmental aplasia, hydrometra

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and mucometra, or hydrocolpos. Diagnosis of these conditions is relatively straightforward with vaginoscopy and palpation.

Male

Similar causes of infertility are identified in these animals as in other species (Pearson et al. 2014d). They can be challenging to diagnose and treat, particularly given the limitations on semen collection in these species in which ejaculation occurs throughout the mating process with the male's legs firmly locked around the female's. Tables 37.1 and 37.2 demonstrate normal characteristics of camelid sperm.

Infertility examinations can be challenging and will require sedation and sometimes general anaesthesia. Failure of penile protrusion can be seen in males younger than 3 years of age due to the normal preputial attachment; however, this is regarded as abnormal if it persists after this age, although it may be an acquired adhesion (Cordero 2008). Trace element deficiencies of copper, zinc, and selenium have been associated with derangements in spermatogenesis (Waldridge & Pugh 1997). The prevalence of testicular hypoplasia is relatively high (10%) and is usually bilateral. Unilateral cryptorchidism is reported in 3% to 6% of male animals (Sumar 1983). Orchitis is usually caused by an infection due to a bite from another male, although it can be caused by haematogenous spread. Affected animals are systemically ill and are in pain. A diagnosis is made on palpation and ultrasonography. Treatment involves analgesia and management of SIRS. Medium term, assuming only one testis is involved, hemicastration will help preserve fertility as the affected testis will undergo degeneration.

Reproductive Biotechnology Techniques

Assisted reproductive techniques are now used in these camelids, although it has been much slower compared with many other species. Further information relating to these techniques is discussed in detail in Chapter 44.

TABLE 37.1 Characteristics of camelid semen

Parameter	Arabian	Bactrian	Llama	Alpaca
Volume (mL)	5–20	1–10	1–5	1–5
Colour	Creamy white to greyish white	Creamy white to greyish white	Milky white	Milky white
Consistency	Highly viscous	Slightly viscous	Highly viscous	Highly viscous
pH	7.0–8.0	7.2–8.0	7.8–8.3	7.2–8.0

Camels

There are two species of Old World camels (Genus *Camelus*), namely *Camelus dromedarius* (one-humped Arabian camel) and *Camelus bactrianus* (two-humped Bactrian camel). All members of the Camelidae family have 74 chromosomes, with a very similar morphology. It has been suggested that *Camelus dromedarius* and *Camelus bactrianus* are two subspecies that descended from the wild Bactrian camel *Camelus ferus* (Larson & Ho 2004). Camels are reared for meat and milk production and for racing. The adult Arabian camel weighs between 300 and 1100 kg. These animals are primarily found in sub-Saharan and North Africa, the Middle East, and the Indian subcontinent. The adult Bactrian camel weighs 300 to 800 kg and reaches 2.5 m in height and is mainly found in China, Russia, Mongolia, Iran, Afghanistan, and Kazakhstan. The crossbreeding of Arabian and Bactrian camels has been practised extensively for many years. Hybrids are larger and heavier than their purebred parents. Camels can produce offspring (a hewar) every 2 years, with a total of 8 hewars throughout their breeding life.

Normal Anatomy

Female

Camel ovaries are enclosed within an ovarian bursa. The ovaries of the camel are oval in shape and located on the cranial border of the broad ligament, just lateral to the uterine horns. Their length, width, and thickness range from 2.5 to 6.0 cm, 1.5 to 4.0 cm, and 0.5 to 1.5 cm respectively. The diameter of mature follicle ranges from 1.2 to 2.2 cm, and the mature CL ranges from 1.2 to 3.7 cm.

The most characteristic feature of the camelid uterus is that the left uterine horn is longer than the right. The uterus of the nonpregnant nulliparous female is located within the pelvic cavity, whereas with parity, the uterus extends beyond the pelvic brim into the abdomen. The uterine body is comparatively small, about 2.5 cm in length, which is less than 10% of the total uterine length.

The length of the cervix ranges from 4.0 to 6.5 cm. Because intrauterine ejaculation occurs in camels, the external cervical os can be readily dilated with a finger or catheter. The vagina is about 30 cm in length. The mammary gland comprises four quarters.

Male

The male genital system is similar to that of domestic farm animals (Fig. 37.2), except that they have well-developed bulbourethral glands and ampullae of the vas deferens; the vesicular gland is absent. Camels are distinctive in having 'poll glands', which are located on the back of the neck, near the poll. They are active

TABLE 37.2 Characteristics of camelid sperm

Parameter	Arabian	Bactrian	Llama	Alpaca
Individual motility (%)	40–60	60–80	20–60	20–60
Sperm concentration (million/mL)	100–350	200–300	60–300	60–300
Total sperm (billion/ejaculate)	0.5–6	0.2–3	0.06–1.5	0.06–1.5
Abnormal sperm (%)	5–20	5–20	10–50	10–50

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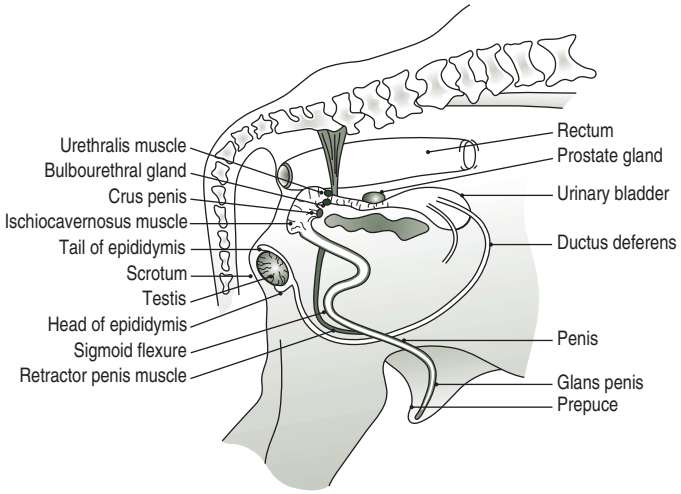
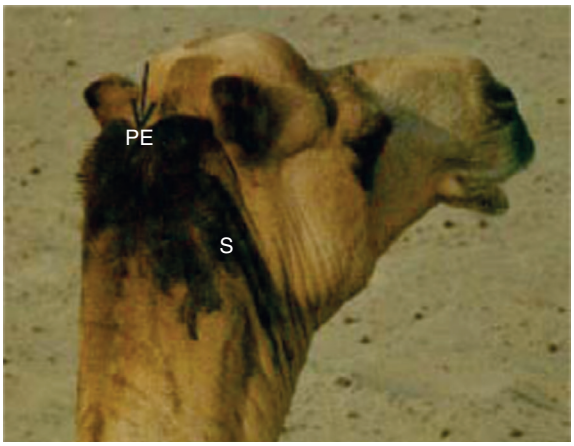


• **Fig. 37.2** Diagram of the reproductive organs of the male Arabian camel. BU, bulbourethral gland; CP, crus penis; DD, ductus deferens; GP, glans penis; HE, head of epididymis; ICM, ischiocavernosus muscle; P, prepuce; PG, prostate gland; PS, penis; R, rectum; RPM, retractor penis muscle; S, scrotum; SF, sigmoid flexure; T, testis; TE, tail of epididymis; UB, urinary bladder; UM, urethralis muscle.



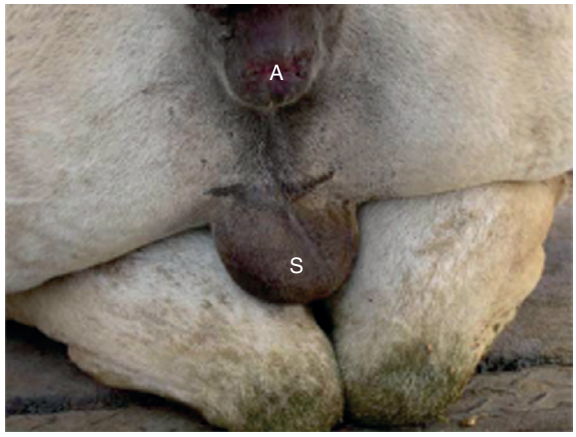
• **Fig. 37.3** The site of the poll gland (PG) in the male Arabian camel. Note how its secretion becomes dispersed over the neck (S).

after sexual maturity and during the breeding season. Their secretion, which runs down the back of the neck, is dark brown in colour and contains a pheromone that is attractive to the female (Fig. 37.3).

The testes of camels, contained within the nonpendulous scrotum, are located in the perineal region, just ventral to the anus, with their longitudinal axes positioned between vertical and horizontal (Fig. 37.4). Testicular descent in camels commences soon after birth and is completed during the second year.

The testes are relatively small compared with other farm animals, weighing 60 to 100 g, but they do increase in size during the breeding season; this is associated with increased sperm output. The average daily sperm production per gram of parenchyma is much lower than that of other farm animals.

The epididymis is located along the dorsal border of the testis with the head curving around the cranial pole. The tail is rounded and prominent. The ductus deferens widens towards the pelvic urethra to form the ampulla. The penis is fibroelastic with



• **Fig. 37.4** Subanal perineal location of the scrotum in the camel. A, anus; S, scrotum.

a prescrotal sigmoid flexure. Separation of the penis from the prepuce at puberty is complete by the second or third year. The nonerect penis is directed caudally, so urination is directed that way. The glans penis is hook-shaped. The urethral orifice opens on the left side of the glans penis (Sumar 2000).

Safe Restraint for Reproductive Examination

Camels are restrained in sternal recumbency using a selection of ropes to facilitate clinical examination of the reproductive system; two short ropes are used to maintain flexion of both carpal joints (to prevent standing), and a long rope is attached to both hind fetlocks that passes over the vertebral column to prevent kicking.

Puberty, Sexual Maturity, and Breeding Season

Female camels reach puberty at around 3 years of age (Chen & Yuen 1979), with sexual maturity occurring at about 5 years of age, although some will show sexual interest by the second year.

Both Arabian and Bactrian camels are regarded as seasonal breeders, with a relatively short breeding season in the cooler months when grazing is optimal (Chen & Yuen 1979). Although camels live peacefully together out of the breeding season, during the breeding season males are hostile to each other (Ismail 1988).

Details on the timing of the breeding season in the Arabian camel are conflicting and vary with geographical region (Al-Eknah 2000). The breeding season in the Bactrian camel is shorter, occurring from mid-January to mid-April in China, for example (Tibary & Memon 1999). It is preferable to breed female camelids early in the season, so that calving occurs before the end of the subsequent breeding season.

Decreasing day length appears to be the stimulus to seasonality. In those living near the equator, there is no influence of photoperiod-induced seasonality, and factors such as rainfall, nutrition and management exert an influence.

Ovarian Activity, Mating, and Ovulation

Camels are induced ovulators. Follicular growth occurs in regular waves during the breeding season (Musa & Abusineina 1978,

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• **Fig. 37.5** A male Bactrian camel displaying flehmen. Note the elevated upper lip *(arrow)*.

• **Fig. 37.6** Copulation in the Arabian camel. Note that the female (cow) is recumbent.

Skidmore et al. 1996). Cyclical follicular activity can be divided into three distinct phases described as the growth phase, the mature phase, and the regression phase. There are some variations, but the duration of these three phases are 10.5 ± 0.5, 7.6 ± 0.8 and 11.9 ± 0.8 days, respectively, in the Arabian camel (Skidmore et al. 1996). The same authors observed that, in about 50% of follicular wave cycles studied, the mature follicle reached a diameter of 2.0 ± 0.1 cm before it regressed. However, in other cycles studied, the mature follicle continued growing over 18.4 ± 0.8 days, before reaching a maximum diameter of 4.1 ± 0.2 cm and, after persisting for 4.6 ± 0.5 days, finally regressing after 15.3 ± 1.1 days. The interval between two successive mature follicular phases ranges between 14 and 28 days. The duration of oestrus is dependent on whether mating occurs, lasting 3 days if it does but up to 7 days if it does not.

On transrectal palpation the uterus feels tonic and firm during oestrus with the presence of one or more mature follicles. In the Arabian camel, maximum oestradiol concentrations in the peripheral blood are about 40 pg/mL when the follicle reaches about 1.7 cm in diameter and, even though it will continue to grow, concentrations fall to basal values of 20 pg/mL. In the absence of ovulation, progesterone concentrations remain low (> 1 ng/mL). After mating and ovulation, the concentrations gradually increase, reaching maximum values close to 3 ng/mL after 8 or 9 days. In the absence of pregnancy, they decline to basal concentrations by Day 11 to 12 (Skidmore 2003).

Female camels do not usually exhibit overt signs of oestrus other than acceptance of the male when hand teased or when they observe other camels mating. Some females may show signs of a mucus discharge from the vulva.

Mature male camels in the breeding season may break out of enclosures or disrupt their tethers to seek out females in oestrus. When a male confronts a female in oestrus, or a competing male, he will extrude the soft palate while making a gurgling sound, spray urine to mark his territory, release the poll gland secretion, and grind his teeth. The Bactrian male camel does not extrude the soft palate but froths from the mouth. The male camel will seek out females in oestrus and will sniff the vulva, perineum, and urine. Pheromones in the urine of females in oestrus trigger the flehmen response in the male (Fig. 37.5); this is a behavioural olfactory response in which he fully extends the head and neck while raising the upper lip and making shallow respiration movements.

Mating occurs with the female in sternal recumbency, and the male squatting behind her with his hind legs flexed and his forelegs extended on either side of her (Fig. 37.6). The duration of copulation varies and tends to decrease as the weather becomes warmer. On average, the Arabian camel mates for 5.5 minutes (range 3–25 minutes).

Females in oestrus quickly respond to the male by becoming recumbent. However, some females, especially inexperienced females or those exhibiting sire preference, will not accept a particular male. The male will attempt to induce recumbency by pressing on the female's neck. After successful courtship, the male squats on the female, pushes himself towards the perineum of the female, and directs his penis forward to seek out the vulva. Partial erection occurs after the female is mounted, and the penis is not fully extended until after intromission is complete. Ejaculation occurs several times during copulation, when the male stretches his entire body and extends his neck.

As camels are induced ovulators, ovulation is triggered by mechanical stimulation of the female genital tract, and in the Bactrian camel, an ovulation-inducing factor has been identified in seminal fluid. This substance has been shown to be a protein or polypeptide with gonadotrophin-releasing hormone (GnRH)-like activity (Zhao et al. 1994, Pan et al. 2001) that will induce ovulation when seminal fluid is placed deep intravaginally or administered by intramuscular injection. Interestingly, in the Arabian camel it does not induce ovulation. Skidmore et al. (1996) have demonstrated that in the Arabian camel the follicle sizes of 1 to 1.9 cm are optimal for ovulation to occur.

Pregnancy and Its Diagnosis

Methods of pregnancy diagnosis described in South American camelids can also be used in camels.

Although camel ovaries are almost equally active, the embryo and fetus will only develop in the left uterine horn, although the right horn is involved in the formation of the placenta. Embryos migrate from the right horn via the uterine body into the left horn, where implantation occurs. The embryo enters the uterus from the uterine tube at 6 to 7 days after ovulation. Maternal recognition of pregnancy has to occur earlier than in other domestic species (before Day 8; Skidmore 2003), when the signal is from synthesis of oestrogens by the embryo as in the horse and pig. The amnion

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develops from Day 14 and the allantochorion is formed by Days 24 to 28 postovulation.

The length of gestation in ranges between 375 and 415 days; this variation in gestation length is influenced by husbandry, number of matings during oestrus, parity of the dam, gender of the fetus, and nutritional status.

Multiple births are uncommon in camelids with a prevalence of 0.4% (Musa & Abusineina 1978). This is of interest as the prevalence of twin ovulations is 14%, of which most are fertilised, suggesting that early death of the second embryo is very common.

The peripheral plasma progesterone concentrations in the nonpregnant camel are low. After mating, at least one CL is formed, secreting a significant amount of progesterone, and values higher than 1 ng/mL are found during pregnancy. During midgestation a rise in oestrogen concentrations is due either to continued follicular development during pregnancy, or secretion from the placenta, or both. High concentrations of oestrogen are present in allantoic fluid on the day of parturition.

The cervical plug provides an important physical seal of the external cervical os during pregnancy. The physicochemical properties of the cervical mucus are related to increases in the plasma progesterone concentrations, resulting in a parallel increase in the protein, alkaline, and acid phosphatase concentration in the mucus (Al-Eknah et al. 1997). A decrease in the degree of hydration, which is under the influence of progesterone, leads to either mucus concentration or alteration in glycoprotein and other cellular proteins, forming linkages affecting the elasticity of the mucus.

Pregnant females do not usually need special care. Frequent handling and particularly rough vaginal examinations performed by camel owners can lead to early embryonic death or abortion. Males should not be allowed access to females during the third trimester of pregnancy because placenta-derived oestrogens produced at that time may suggest that the pregnant female is in oestrus.

Differential Diagnoses for Pregnancy

A cyst-like structure formed at the infundibulum of the uterine tube of the Arabian camel can be confused with pregnancy (Ali et al. 1992) because its weight pulls the uterus over the pelvic brim and into the abdomen, thus resembling pregnancy. Ultrasound of the cyst will identify a large anechoic structure, but no embryo or fetus will be identified.

Pyometra is common in the nonpregnant Arabian camel because of the frequent and unhygienic vaginal examinations carried out by owners and the copulatory behaviour of the male camel. On transrectal palpation, it usually resembles a 2- to 3-month pregnancy (Enany et al. 1990). Ultrasonography reveals a largely hypoechoic mass with anechoic and hyperechoic areas but with no embryo or fetus. Hydrometra and mucometra occur occasionally, associated with a persistent hymen in nulliparous individuals or vaginal adhesions in parous females due to the accumulation of fluid because of impaired drainage.

Parturition and Postpartum Period

Signs of approaching parturition include separation from the herd, restlessness, relaxation of the sacrosciatic ligaments, and swelling of the vulva accompanied by a vulvar discharge resulting from liquefaction of the cervical plug. Appetite may be normal until an hour or so before the start of labour. Some of these premonitory signs may commence as early as 15 days before labour.

The first stage of labour is characterised by increased myometrial contractility and complete relaxation and passive dilatation of the cervix. The animal may look at her flank, grind her teeth, increase the frequency of urination and defecation, stand up and lie down, move around, and raise the tail. The first phase lasts 4 to 40 hours and is usually longer in primiparous than multiparous females. Camel dams give birth at any time of the day or night.

The appearance of the fetus enclosed within its membranes at the vulva and the presence of regular straining indicate the onset of the second stage of labour. The dam is very restless, standing up and lying down in either sternal or lateral recumbency. However, the fetus is normally expelled with the dam standing. The second stage of labour normally lasts 0.25 to 1 hours. The expulsion of the fetal membranes (third stage of labour) usually takes less than 1 hour. Camels do not lick the newborn or eat the afterbirth.

On the third day postpartum, the uterus in camels cannot be easily palpated transrectally because of its size. The uterine wall is still thickened with longitudinal rugae. Ten to 15 days after birth, the uterus can be readily palpated and involution is complete by 16 to 20 days postpartum. By 16 to 20 days postpartum, the cervix appears closed but with some congestion of the cervical folds, and is involuted by Day 26 to 30.

Camels normally have a long anoestrus after parturition if they naturally rear their calves, although if well fed, they can come into oestrus. This will certainly occur if the calf dies or is weaned.

Care of the Dam and Newborn

High mortality rates (> 25%) among newborn hewars have been reported, especially during the first 3 weeks of life, with causes including failure of passive transfer, sepsis that may manifest as diarrhoea, cold shock, and heat stress (Bustinza et al. 1988). Traditionally, the Bedouin do not breed in the late season to avoid the dams calving during the hot season.

Dystocia

There is a scarcity of published information on the prevalence of dystocia in camels. Bedouin camel owners report a familiarity with the recognition and treatment of dystocias due to abnormal disposition, such as carpal flexion, lateral deviation of the head and hock, and hip flexion, although posterior presentation is uncommon (Al-Eknah, personal communication). Fetopelvic disproportion, monsters, and transverse presentations are rare, and the frequency of twin births is low (0.1%–0.4%). In one study from Saudi Arabia, 43% of dystocias were due to maternal factors and 57% due to fetal factors. Of the maternal causes, uterine torsion was the most common (50%), then primary uterine inertia (20%) and incomplete cervical dilatation (20%), and vaginal and vulvar stenosis in a much smaller number of animals (10%; Aboul-Fadle et al. 1993). In the same study, abnormal fetal posture was responsible for 90% of fetal causes.

Under field conditions, the correction of limb and neck flexions, in which substantial fetal retropulsion is required, can be facilitated by casting the dam, front end first, in a deep pit or on a slope excavated in the sand, so that the rear end is raised. Head and limb extension is more difficult to achieve than in the cow. Limited evidence indicates that the camel fetus survives dystocia better than the equine fetus and that the camel is a good subject for Caesarean section. When the fetus is dead, fetotomy can be successful. For a Caesarean operation, the camel is cast on her right side under xylazine sedation, and the operation is performed

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under regional infiltration of local anaesthesia with the incision made in the caudal aspect of the left sublumbar area.

Disorders and Diseases of Pregnancy

Pregnancy failure is relatively common in camels. Early embryonic death and abortion can be as high as 40% and 20%, respectively.

Conception Failure

Fertilisation failure and early embryonic loss are common in Arabian camels. The main causes of these repeated returns to oestrus after mating are usually associated with abnormalities of the uterus or uterine tubes.

Fetal Mummification

This has been reported in camels. The sterile death of a well-developed fetus may be followed by persistence of the CL, with resorption of the fetal fluids and soft tissue, leaving a fetal mummy within the uterus.

Abortion

Infectious and noninfectious abortions are commonly identified in camels as they are in other species. Of concern is Q-fever, caused by *Coxiella burnetii,* which is a well-established cause of abortion in camels and presents serious risks to human health (Tibary 2006). Abortions have been reported in camels infected with rift valley fever in which no other clinical signs were seen. Antibodies against bluetongue virus have been reported in camels in Sudan and in Saudi Arabia. However, no clinical signs of abortion were observed.

Uterine Torsion

This is also a common problem in camels and usually requires surgical correction through a flank laparotomy.

Postpartum Problems

Uterine Prolapse

This is a common complication postpartum; Fig. 37.7 demonstrates a vaginal prolapse. Replacement under caudal epidural is very similar to that described in the cow.

Infertility in Camels

The reproductive rate in camelids is moderate to low (Gordon 1997). This was shown by studies of 30 herds in Tunisia, where there was a calving rate of 40% and a mortality rate between birth and 1 year of 17% (Djellouli & Saint-Martin 1992). However, anecdotal evidence from some Bedouins is that, for every 100 Arabian camels mated, 80 to 90 produce calves. About 1% of the camels are sterile. Their fertility is maintained throughout life, normally breeding in alternate years; a female can over her breeding life yield a total of 12 offspring, although an average of eight is more likely. One mating per oestrus is usual, and it is possible for a male to serve five or six females in a day. It is said that one male is sufficient for 200 females with controlled breeding, but a much smaller number is customary. Both male and female factors are responsible for camel subfertility.

• **Fig. 37.7** Vaginal prolapse in an Arabian camel, as indicated by the *arrow*.

Female

Congenital Anatomical Defects

Ovarian hypoplasia and agenesis, persistent hymen, and freemartinism have been reported in Arabian camels.

Acquired Anatomical Defects

Parturient trauma of the tubular genital tract, vaginal adhesions, and perineal rupture are the most common, whereas neoplasia of the genital organs occurs rarely. Cyst-like structures have been associated with the infundibulum of the uterine tube (Ali et al. 1992).

Follicular cysts are common in camels (El-Wishy & Hemeida 1984), resulting in nymphomania; they can be treated with GnRH or human chorionic gonadotrophin (hCG).

Early embryonic death with persistence of the CL can cause pseudopregnancy, which can be successfully treated with $PGF_{2\alpha}$.

Environmental Factors

Adult camels are well adapted to extremes of both heat and cold, although the newborn are susceptible to hyper- and hypothermia. The former can be avoided by providing shelters with water sprinklers and the latter by providing deep straw bedding and a dry shelter with protection from the wind.

Poor Management

The absence of breeding records can be considered one of the most important management deficiencies that results in subfertility in a herd. In addition, many camel owners do not have a strategy for selection for good fertility or for culling those that are subfertile.

Nutrition

Nutrition plays a vital role in the various physiological events leading to the attainment of sexual maturity and subsequently normal reproduction. The faster the growth rate, the earlier sexual maturity occurs. It has been found in Kenya that dam nutrition and health status have an influence on birth weight of the hewars (Wilson 1986). Undernutrition delays puberty in both young males and females and, if severe, might cause retrogressive changes in the reproductive organs after they are fully developed (Tibary and Anouassi 1997).

Infectious Agents

Metritis and endometritis is an important cause of infertility and subfertility in camelids. This can be ascribed to the following predisposing factors:

The copulation process: With the female squatting on the ground during coitus for up to 25 minutes, there is significant opportunity for the penis to transfer debris and bacteria

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• **Fig. 37.8** Vulvar discharge in an Arabian camel *(arrow)* after the surgical breakdown of vaginal adhesions.

• **Fig. 37.9** Bilateral cryptorchism in an Arabian camel. (A) Anus and (SC) scrotum with no testes present.

from the immediate environment into the uterus. This is exacerbated by repeated matings.

Unhygienic and rough intravaginal and intrauterine examinations performed by camel owners: It is believed by Bedouins that the reason for a female camel failing to conceive is incomplete intromission. As a consequence, many camel females are subjected to gentle and gradual digital dilatation of the cervix, using butter as a lubricant. After this treatment, complete intromission is said to be possible and pregnancy ensues.

Dystocia and postpartum complications: These may result in the formation of cervical and vaginal adhesions, which can interfere with uterine drainage, resulting in the accumulation of up to 15 litres of fluid or purulent material in the uterus (Fig. 37.8). Uterine infection may result in endometritis or pyometra.

Male

Male camel fertility is generally good. One male Arabian or Bactrian camel can serve up to 50 females in one breeding season. Camelid semen is characterised by the absence of mass sperm motility and the presence of gel to which the sperm are attracted (Rai et al. 1997). Liquefaction of the highly viscous semen can take place within 60 minutes at 37°C. However, if liquefaction is delayed for more than 1 hour, sperm longevity is affected. Tables 37.1 and 37.2 demonstrate the characteristics of camel sperm.

Infertility in the male can be due to poor libido, which is uncommon. Causes of inability to copulate are similar to those described in other species. One disorder peculiar to Arabian camels is trauma to the prepuce and penis due to biting injuries after fights, which may lead to penile adhesions that need surgical separation. Cryptorchidism is relatively common in camels (Fig. 37.9).

Semen Collection

Semen collection in camels is difficult due to mating occurring with the female recumbent in a sitting position (see Fig. 37.6); it can last up to 25 minutes, and during coitus, there is interlocking of the hindlimbs of male and female.

Several semen collection techniques described for other species have been modified and adapted, all with varying degrees of success. The two main methods used are the artificial vagina (AV) and electroejaculation. Collection of semen from the camel using electroejaculation requires sedation and special restraint. The ejaculate is of low volume and poor in quality, and there is the possibility of contamination with urine and cellular debris.

• **Fig. 37.10** Collection of semen from a Bactrian camel using an artificial vagina. Note that with both cow and bull recumbent the operator has to squat on one knee.

The AV is considered the most suitable means of collection (Fig. 37.10), with several studies undertaken to improve the method. Because the male camel in the breeding season can be vicious and aggressive, the most important consideration in designing facilities for semen collection is the safety of the handler and collector.

El-Hassanein (2003) described semen collection in dromedaries using an AV housed inside a dummy female. Al-Eknah et al (2001) devised a safer and more convenient method of collecting semen from the male dromedary, in which there was an excavated pit beneath the collection area where the person collecting the ejaculate using an AV could be positioned. This enabled appropriate positioning of the AV beneath the teasers' perineum and alignment with the base of the penis so that semen collection was relatively easy. In addition, ventral or lateral bending of the penis was avoided, allowing better semen discharge as well as providing the collector with a better view of the mating process and ejaculation. The main disadvantage of using such a facility, or a dummy to which the AV is attached, is the requirement for the male to be trained. However, once this is achieved, collection is rapid and efficient.

Semen used in artificial insemination (AI) programmes should be of high quality, and collected from males in good health and nutritional status.

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For preservation of camel semen, different extenders have been used, including Laiciphos, Androlep, sodium-citrate-egg yolk, Dimitropolous extender, Tris-egg yolk, and green buffer extenders (Bravo et al. 2000).

One of the obstacles for AI in camels is the relatively short life span of sperm in extended semen of 1 to 6 hours at 4°C.

Superovulation and Embryo Transfer

The small number of offspring likely to be produced by a prestigious female camel in her relatively short breeding life restricts the distribution of her desirable genetic material. Because of the restricted breeding season and the long gestation period, judicious use of embryo transfer can increase reproductive efficiency.

The optimal response to superovulatory treatment occurs after a period of progesterone priming of the ovaries. In farm animals, unlike camelids, the CL formed after spontaneous ovulation provides the progesterone priming. Thus to induce superovulation for embryo transfer, gonadotrophin is usually injected during the luteal phase, which is then shortened, 24 to 48 hours later, by the administration of a luteolytic dose of $PGF_{2\alpha}$. Because camels are induced ovulators and only have CLs after mating, it is possible that the progesterone-dominated environment of dioestrus, which is thought to be necessary to provide priming for the wave of follicular growth in spontaneously ovulating species, may not be necessary in camels.

For superovulation, equine chorionic gonadotrophin (eCG) has been used successfully to stimulate the ovaries to produce multiple follicles. However, higher doses of eCG may hyperstimulate the ovaries, resulting in the formation of follicular cysts with a diameter of more than 2.5 cm. Another method for superovulation involves the use of ovine follicle stimulating hormone (FSH) in a split dose regime over 3 to 6 days, which gives better results but is time consuming. Combined treatment with eCG and FSH has given more promising results. Recently, studies using priming with GnRH followed by eCG and/or FSH (Ismail et al. 2007) have been successful. The gonadotrophins are given just before or after the removal of the PRID or CIDR or on the last day of other progesterone therapy. Exogenous gonadotrophins may also be administered during a luteal phase created by mating the female with a sterile male.

The superovulatory response to exogenous gonadotrophin therapy varies between individuals, with embryo recovery per donor camel ranging from 0 to 30, with a mean of six per donor (Skidmore & Adams 2000).

Breeding After Superovulation

In order to achieve good superovulation rates, donors should be monitored using ultrasonography and palpation throughout the treatment regime and bred when the follicles reach a suitable size of between 13 and 16 mm.

To ensure optimum fertilisation rates of superovulated animals, a single injection of GnRH or hCG at the first mating or insemination should be given. Mating or insemination should be repeated 12 hours later. Ovulation failure can occur if animals are bred too early or if there is low luteinizing hormone (LH) response.

Flushing of Embryos

Donors are flushed 6 to 9 days from the first mating. The embryos recovered on Day 7 range from compact morulae to expanded blastocysts. Both surgical and nonsurgical embryo recovery have been performed in camels.

The type of superovulatory treatments used can also affect the embryo recovery rate. eCG treatments usually result in low recovery rates, probably due to an increase in spontaneous luteinisation associated with the high LH activity in eCG and its long half-life. The time of initiation of the superovulatory treatment also affects the embryo recovery, with the best results obtained when treatment is initiated in absence of any follicle growth. The time of flushing may also affect embryo recovery because the suggested optimum time for flushing dromedary camels is Day 7 to 9 after mating and treatment with GnRH.

Management of Recipients and Transfer of Embryos

For immediate transfer of embryos, the ovarian activity of the recipient females should be synchronised with that of the donor female. It is recommended that embryos should be transferred on Day 6 after ovulation in recipients.

Collected embryos from donors at Day 7 to 9 are generally at the hatched blastocyst stage. Embryos used for transfer should be morphologically normal. Ovulation is induced in the recipient females by the use of vasectomised males or by the injection of hCG or GnRH.

Freshly collected embryos can be stored for a few hours before transfer in DPBS media. However, long-term storage requires freezing and the use of the associated technology described for other species.

Each embryo is loaded into a 0.25 ml AI straw and, using a modified Cassou insemination gun, is introduced transcervically into the left uterine horn, irrespective of whether the CL is on the ipsilateral or contralateral ovary.

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38

Buffalo and Related Species

OSWIN PERERA AND DAVID E. NOAKES

The domestic water buffalo (*Bubalus bubalis*) is an important livestock resource in many countries of Asia, the Mediterranean region, and Latin America (Central and South America). It plays an important role in providing milk, meat, and draught power in agricultural systems, particularly in the developing countries. The world population of water buffalo is estimated to be 194 million (FAO 2017), of which 96% are in Asia. They are classified into two main 'types' based on their preferred wallowing environment, namely running water for the river type and stagnant pools for the swamp type (Cockrill 1974). The Mediterranean buffalo, which some consider to be a third type, is derived from the river type. Systems of buffalo production vary widely through the different regions of the world (Perera et al. 2005) and are determined by several interacting factors that include climate (tropical or temperate, humid or arid), location (rural, periurban, or urban), cropping system (rain-fed or irrigated), type of operation (small or large scale, subsistence or commercial), and primary purpose (milk, meat, draught, or mixed).

The river buffalo was originally restricted to the South Asian region, has a chromosome configuration of 50, and is reared mainly for milk production. The swamp buffalo originated in Southeast and East Asia, has a chromosome configuration of 48, and is used mainly for draught purposes and meat. In these regions, buffaloes are kept mostly by small scale producers, who raise a few animals in mixed crop–livestock systems. The Mediterranean type predominates in the Middle East, North Africa, and Europe, where they are reared for milk and meat under a wide range of farming systems, including modern dairy farms as in Italy. All types of buffalo have spread beyond their traditional home regions, most notably to Latin America, where they are raised in farms ranging from small to very large scale and under systems ranging from intensive stall-fed to extensive grazing.

The main breeds of dairy buffalo belong to the river type and include the Murrah, Surti, Jafarabadi, and Nili-Ravi. The swamp type has no specialised breed, but selective breeding in some countries has resulted in populations with characteristic features. Crossbreeding between the types has been done in many countries, with the objective of combining the higher milk yield of the river buffalo with the better adaptability and working ability of the swamp buffalo. Despite their different karyotypes, the crossbreeds are fertile (Fischer & Ulbrich 1967). The hybrid female buffalo produces viable calves and the hybrid male, despite the presence of a high percentage of degenerating spermatocytes and abnormal spermatids in the testes (Bongso et al. 1983), appears to have normal fertility.

Although buffalo can adapt to harsh environments and live on low quality forage, their reproductive efficiency is often compromised by such conditions (Perera 2008). Various factors, including climatic stress, depress ovarian cyclicity, oestrous expression, and conception rates. Poor nutrition, often related to seasonal fluctuations in availability and quality of feed, delays puberty and increases the duration of postpartum anoestrus. Management factors, such as the system of feeding (free grazing, tethered, or stall-fed) and sucking by calves (restricted or *ad libitum*) also modulate reproductive functions. The relative importance of these factors varies greatly depending on ecological conditions and production systems. However, studies in Sri Lanka (Perera et al. 1987) and Brazil (Vale & Ribeiro 2005) show that they can have good fertility, provided that genotypes are matched to the environment, and they are managed and fed properly. The main reproductive characteristics of buffalo, based on reports from several countries on river and swamp types kept under a wide range of management systems, are summarised in Table 38.1 (adapted from Perera 2008).

Reproduction in the Female

Anatomy of the Reproductive Organs

The reproductive organs of the buffalo are basically similar to those of cattle, with some important differences. The vulvar labia are less tightly opposed, and the clitoris is more developed. The uterus and cervix are smaller than those in cattle, and the nonpregnant tract lies wholly within the pelvic cavity, with the uterus usually being tightly coiled. The cervical canal is narrower than that in cattle and is more difficult to negotiate during artificial insemination (AI), especially in heifers. The ovaries of cyclic buffalo heifers have a reservoir of only 10,000 to 20,000 primordial follicles, compared with over 100,000 in cattle (Danell 1987). The mature ovaries are smaller than those in cattle, weighing around 2.5 g when inactive and 4 g when active, with fewer tertiary follicles (Zicarelli 1997). Mature follicles range from 10 to 16 cm in diameter, tend to protrude from the surface of the ovary, and can be mistaken for an early corpus luteum (CL). The CL is smaller than that in cattle, ranging from 10 to 17 mm in diameter, often does not protrude markedly from the surface of the ovary, and sometimes lacks a clear crown (Fig. 38.1). These characteristics make accurate identification of ovarian structures by rectal palpation in buffalo more difficult than in cattle (Perera et al. 1987).

Puberty

Buffalo heifers usually attain puberty when they reach about 55% to 60 % of their adult body weight, but the age at which they attain

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TABLE 38.1 Summary of reproductive characteristics of buffalo

Parameter	Mean	Range
Age at puberty (months)	30	16–46
Weight at puberty (kg)	275	200–350
Length of oestrous cycle (days)	21	17–25
Length of oestrus (hr)	10	5–27
Time of ovulation:		
- After onset of oestrus (hr)	34	24–48
- After end of oestrus (hr)	14	6–21
Length of gestation (days):		
- River type	310	300–320
- Swamp type	330	320–340
Birth weight of calves (kg)	26	22–36
Involution of uterus (days)	30	25–35

From: Perera 2008.

• **Fig. 38.1** Ovaries from a buffalo cow, with the right ovary containing a corpus luteum that does not protrude markedly from the surface of the ovary and lacks a clear crown.

puberty can be highly variable, ranging from 16 to 46 months (Jainudeen & Hafez 1993). The factors that influence this are genotype, nutrition, management, social environment, climate, year or season of birth, and diseases. A review of studies from many countries (Borghese 2005) shows that, under favourable conditions, river types exhibit their first oestrus at 16 to 18 months, whereas swamp types do so at 21 to 24 months. The body weight at which puberty is attained is strongly influenced by genotype and is around 200 to 300 kg for swamp types and 250 to 350 kg for river types. Although buffalo attain puberty later than cattle, they have a longer reproductive life, which tends to compensate for this early economic disadvantage.

Seasonal Changes in Breeding Activity

Buffalo are polyoestrus and are capable of breeding throughout the year. However, in many countries, a seasonal pattern of breeding activity, and consequently of calving, has been observed. In tropical locations where photoperiod is relatively constant, annual changes in rainfall appear to influence cyclicity, acting through the availability and quality of herbage (Perera et al. 1987). In regions where hot summer months cause heat stress, acyclicity is associated with high prolactin concentrations in the blood, which is thought to influence ovarian activity as well as causing subfertility and repeat breeding by lowering progesterone secretion (Roy & Prakash 2007). In temperate regions such as Italy, where buffalo are fed with a constant balanced diet and are not exposed to heat stress, a distinct seasonal reproductive pattern is also found. This has been attributed to changes in photoperiod, mediated by melatonin secretion (Borghese 2005).

The Oestrous Cycle

The duration of the oestrous cycle in buffalo is from 17 to 25 days with a mean of around 21 days and, compared with cattle, is more variable with a higher incidence of both abnormally short and long cycles. The duration of oestrus or 'standing heat' varies between 5 and 27 hours, and ovulation occurs about 24 to 48 hours (mean 34 hours) after onset of oestrus, or 6 to 21 hours (mean 14 hours) after the end of oestrus (see Table 38.1). In hot climates the duration of oestrus tends to be short, and the signs may be exhibited only during the night or early morning. In Italian dairy buffalo, Zicarelli (1997) has recorded different durations of oestrus, categorised as short (< 12 hours), medium (13–24 hours), long (24–48 hours) and very long (> 48 hours).

Cyclical Changes of the Genitalia and Reproductive Hormones

Ovaries

During the oestrous cycle, waves of follicular recruitment, growth, and regression occur as in cattle. The number of follicular waves per cycle range from one to three, resulting in differences in the mean length of the luteal phase and the interval between ovulations (Baruselli et al. 1997). Heifers tend to have cycles of two follicular waves, resulting in shorter interoestrous intervals compared with cows (21 versus 24 days) (De Rensis & López-Gatius 2007). The mean diameter of follicles at the time of the deviation between dominant and subordinate follicles is 6.2 and 5.8 mm, respectively, and is larger than in zebu cattle but smaller than in taurine cattle. During oestrus, a mature follicle can be palpated transrectally or imaged by transrectal ultrasonography as a turgid area protruding slightly from the surface of the ovary; follicles up to 15 mm associated with the nonovulatory waves can be identified for most of the rest of the oestrous cycle. On the day of ovulation (Days 1–2), the follicle softens, and the site of ovulation is felt as a pit or depression on the surface of the ovary. Normally, one oocyte is shed per cycle. The developing CL (Days 2–7) is soft and difficult to palpate per rectum, but the mature CL (Days 8–16) is palpable as a firm structure, which may or may not protrude from the surface of the ovary (see previous mention in Anatomy section). In the absence of pregnancy, the CL regresses (Day 17) and becomes firmer, and old CLs appear as white scars on the surface of the ovary. Buffalo CLs have no yellow coloration at any stage of development due to lower amounts of beta carotene.

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Hormones

The changes in concentrations of progesterone in blood and milk during the oestrous cycle are similar to those in cattle, but the peak concentration is relatively lower (Perera 1999, Singh et al. 2001). Progesterone concentration in fat-free milk is usually less than 1 nmol/l (~ 0.3 ng/mL) during the follicular phase, and ranges from 3 to 12 nmol/l (~ 1–4 ng/mL) during the luteal phase and pregnancy. For assessing cyclic ovarian activity and for early diagnosis of nonpregnancy, progesterone concentrations greater than 3 nmol/l are considered to be indicative of the presence of luteal function, and those greater than 1 nmol/l are considered indicative of the absence of luteal function, with intermediate values being considered inconclusive. The concentration of oestradiol-17β in blood during the follicular phase of the cycle also appears to be relatively lower than that in cattle (Roy & Prakash 2009). Although this has been suggested as a possible reason for the lower intensity of oestrus exhibited by buffalos, studies on Italian buffalo have shown no differences in the endocrine profiles of those with overt and silent oestrus (Zicarelli 1997). The temporal patterns of both luteinising hormone (LH) and follicle stimulating hormone (FSH) in buffalo are basically similar to those in cattle, with a preovulatory LH surge occurring on the day of oestrus and lasting 7 to 12 hours (Kanai & Shimizu 1986).

Genital Tract

Externally detectable physical changes around the time of oestrus include swelling of the vulva and reddening of the vestibular mucosa. Vulval swelling results in effacement of the horizontal wrinkles that are present on its external surface and this, together with vestibular reddening, can be detected by regular examination of individual animals under confined systems. Mucus secreted from the cervix during oestrus is less copious than in cattle and does not usually hang as strands from the vulva, but it tends to accumulate on the floor of the vagina and be discharged when the animal is lying down. Thus in buffalo that are housed or tethered, heat detection can be aided by examining the floor near the rear of the animal each morning and evening for signs of mucus. Mucus discharge is often noticeable when palpating the genital organs per rectum during oestrus (Fig. 38.2).

The internal tubular reproductive tract undergoes changes similar to those in cattle. The uterine horns are turgid and coiled, with maximum tone during oestrus, and become oedematous at the time of ovulation. They gradually lose their turgidity and tonicity after ovulation to become almost flaccid during the luteal phase of the cycle. The cervix dilates sufficiently during oestrus to enable the passage of an insemination catheter into the uterus. The clear, copious mucus that is secreted during oestrous changes to an opaque, thick, scanty discharge after ovulation. Oedema of the vulva and hyperaemia of the vaginal mucous membrane also subside after oestrus. The 'metoestrus bleeding', often seen in cattle, rarely occurs in the buffalo.

• **Fig. 38.2** Discharge of clear mucus from the vagina during oestrus in a buffalo cow.

Behaviour During Oestrus and Mating

A major difference between buffalo and cattle is that behavioural signs of oestrus are less overt in the former, and homosexual behaviour between buffalo females is rare (Jainudeen & Hafez 1993). In the absence of a bull, the main behavioural signs are restlessness, bellowing, raising of the tail, and frequent voiding of small quantities of urine, but these are not consistently exhibited by all animals. During periods of high ambient temperature, the duration of heat may be shorter and the signs exhibited only during the night or early morning.

Mating behaviour is similar to that in cattle. When a bull approaches a female in oestrus, it exhibits sniffing and licking of the perineum and vulva, as well as a flehmen reaction. In some cases, vocalisation, tucking up of the sheath, and intermittent urination may also be seen. An oestrous female responds by standing immobile for the male to mount and perform intromission. The copulatory behaviour includes penile erection, grasping the female at the level of the pelvis, muscular contractions at the base of the tail, penile movements to locate the vulva, intromission, and an ejaculatory thrust. Mating lasts 20 to 30 seconds. The male dismounts and retracts the penis into the sheath, while the female remains with her back arched and tail elevated for a few minutes.

Methods of Oestrous Detection

Detection of oestrus by observation of changes in the external genitalia and behavioural signs is difficult due to them being less pronounced in buffalo than in cattle. In the smallholder farming systems, improved detection is possible through training and motivation of farmers to undertake regular and close observation, together with proper identification of individual animals and keeping of good records (Perera 1999). In larger farms, a vasectomised teaser bull fitted with a chin ball marking device can be used for oestrous detection. The male is either kept in a corral with females from late evening until the next morning or, if they are in stanchions, led behind them twice daily. If no male is available, a buffalo cow can be androgenised for oestrous detection. Heat detection aids used in cattle, such as KaMaR and tail paint (see Chapter 22), have been used in buffalo herds in conjunction with a teaser bull but are unreliable when wallowing is practised. More advanced automated systems, such as pressure sensing radiotelemetric detectors (HeatWatch system) developed for cattle, could be applicable under improved management systems when the costs can be justified. The use of electronic devices such as pedometers and probes to measure vaginal electrical resistance (Gupta & Purohit 2001) has yielded good results under some conditions. Alternatively, as in cattle (see Chapters 8, 25, 26), various regimens of oestrous synchronisation and oestrous induction have been used in buffalo with the object of removing the need for oestrous detection by using fixed time AI (see later under 'Improvement of Fertility').

Pregnancy

The embryo reaches the uterus by Day 4 to 5 after oestrus, and the blastocyst hatches by Day 6 to 8. As in cattle, the growing

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extraembryonic tissues produce interferon tau, which prevents luteolysis by blocking the production of prostaglandin F2α from the endometrium (Sharma et al. 2014). The duration of gestation in the buffalo is nearly 1 month longer than in cattle and ranges from 300 to 330 days, with a mean of about 310 days for river types and 320 days for swamp types (Perera & de Silva 1985, Vale et al. 1990). River and swamp hybrids have an intermediate gestation length of 315 days. Male calves are carried 1 to 2 days longer than female calves. The incidence of right horn pregnancy is higher than left horn (67% vs 33%), and transuterine migration of the embryo is rare. The synepitheliochorial placenta of the buffalo is of the cotyledonary type. The total number of placentomes in the uterus can vary from 70 to 200, with the gravid uterine horn having a higher number than the nongravid horn (Pawshe & Purohit 2013). In late pregnancy, the placentomes measure 5 to 10 cm in diameter. As in cattle, cyclical ovarian activity ceases during pregnancy, but a few buffaloes may show behavioural signs of oestrus, which is anovulatory. The CL is maintained throughout gestation, and plasma progesterone concentrations remain elevated until parturition. The role of the CL in maintenance of pregnancy during the late stages of gestation is not known.

Pregnancy Diagnosis

The techniques for pregnancy diagnosis are basically similar to those routinely used in cattle and include palpation of the genital tract via the rectum, ultrasonography, and hormone assay (see Chapter 5). An important difference is that buffalo have a longer gestation length than cattle (see Table 38.1), resulting in a slightly slower development of the fetus and corresponding differences in criteria for determining the stage of gestation.

Rectal Palpation

Enlargement of one horn of the uterus can be detected from about 35 days of gestation, and slipping of the allantochorion is possible from about 42 to 56 days. The enlarged uterus is palpable in the pelvic cavity up to the third month of gestation, thereafter descending to the abdomen. In some deep-bellied river buffalo breeds, the fetus may be difficult to palpate, particularly between the sixth and eighth months. In such cases, palpation of the hypertrophied middle uterine arteries, with fremitus, or recognition of the placentomes aids in the diagnosis.

Ultrasound Scanning

Using a 5.0 or 7.5 MHz linear array transducer designed for transrectal use, the conceptual vesicle can be detected as an anechoic area from about 20 days onwards, sometimes with an echogenic embryo (Pawshe & Purohit 2013). The fetal heart can be detected at 30 to 35 days and structures such as limbs, spinal cord, and fetal membranes by 35 to 40 days.

Hormone Assays

As in cattle, elevated progesterone concentrations in milk or plasma 20 to 23 days after breeding is a reliable indicator for the diagnosis of nonpregnancy with an accuracy of nearly 100%, but the test is only 65% to 80% accurate for a positive pregnancy diagnosis (Perera et al. 1980). The inaccuracy in the latter case is due to conditions other than pregnancy that can result in high progesterone levels at this time, such as AI during the luteal phase of the cycle, persistence of the CL due to embryo death after 15 to 16 days of gestation or pyometra, and luteal cysts. Hence rectal palpation at a later stage is necessary to make a definitive diagnosis of pregnancy. The advantage of progesterone measurement is that nonpregnant animals can be identified early, and the farmer alerted to the need for detecting heat and having the animal mated. Measurement of oestrone sulphate in blood or milk can accurately differentiate pregnant from nonpregnant females at 110 days, as reported in cattle (see Chapter 5).

Parturition

The physiological processes leading up to and during parturition, as well as the changes occurring in the main hormones, are similar to those in cattle. Commencing about 1 to 2 weeks before parturition, the female shows marked abdominal enlargement, udder development, and hypertrophy and oedema of the vulvar lips. As the time of parturition approaches, she normally isolates herself from the rest of the herd. The relaxation and sinking of the pelvic ligaments and muscles lead to an elevation of the tailhead, while liquefaction of the cervical seal of pregnancy results in a string of clear mucus hanging from the vulva, particularly when the animal is recumbent.

Plasma concentration of progesterone remains elevated throughout gestation and declines sharply at the time of parturition, whereas the concentration of both oestrone and the main metabolite of $PGF_{2\alpha}$ (PGFM) increase about 15 days before parturition and reach peak values 3 to 5 days prepartum (Perera et al. 1981). There is a significant increase in plasma concentrations of cortisol at parturition (Prakash & Madan 1984).

Stages of Labour

Stage 1 – dilation of the cervix: occurs about 12 to 24 hours before parturition, uterine contractions increase in both frequency and amplitude, causing the animal some abdominal discomfort. The cervix takes about 1 to 2 hours to dilate fully.

Stage 2 – delivery of the fetus (Fig. 38.3): As the fetus enters the birth canal, the dam lies down in sternal or lateral recumbency and starts straining. The allantochorion mostly ruptures before it reaches the vulva and is quickly followed by the fetus contained within the amnion, appearing at the vulva. Strong abdominal contractions lead to the rupture of the amniotic sac and the delivery of the fetus, usually in anterior longitudinal presentation and dorsal position, with extended limbs; posterior presentation is uncommon. This stage of labour lasts 30 to 60 minutes but may extend up to 6 hours, particularly in primiparae. As in the cow, the umbilical cord ruptures before the calf reaches the ground. Twinning is rare; the incidence is less than 1 in 1000 births.

Stage 3 – expulsion of the placenta: After delivery of the fetus, abdominal straining ceases and the fetal membranes are expelled, usually within 4 to 6 hours.

Dystocia

Dystocia is less common in the buffalo than in cattle; its incidence ranges from 2% to 5%. Stabled river buffaloes are more prone to dystocia than the free-ranging swamp type. The commonest cause of dystocia is fetomaternal disproportion, followed by a variety of faulty dispositions. The most frequent cause of maternal dystocia is uterine torsion, followed by incomplete dilatation of the cervix and uterine inertia. Occasional cases of hydrallantois and persistent hymen have also been reported. Most cases of uterine torsion occur at the time of parturition or during the last month of pregnancy. The direction of torsion in the buffalo in more than 90% of cases is to the right (clockwise). Predisposing factors for both torsion of the uterus and prolapse of the vagina or uterus are anatomical

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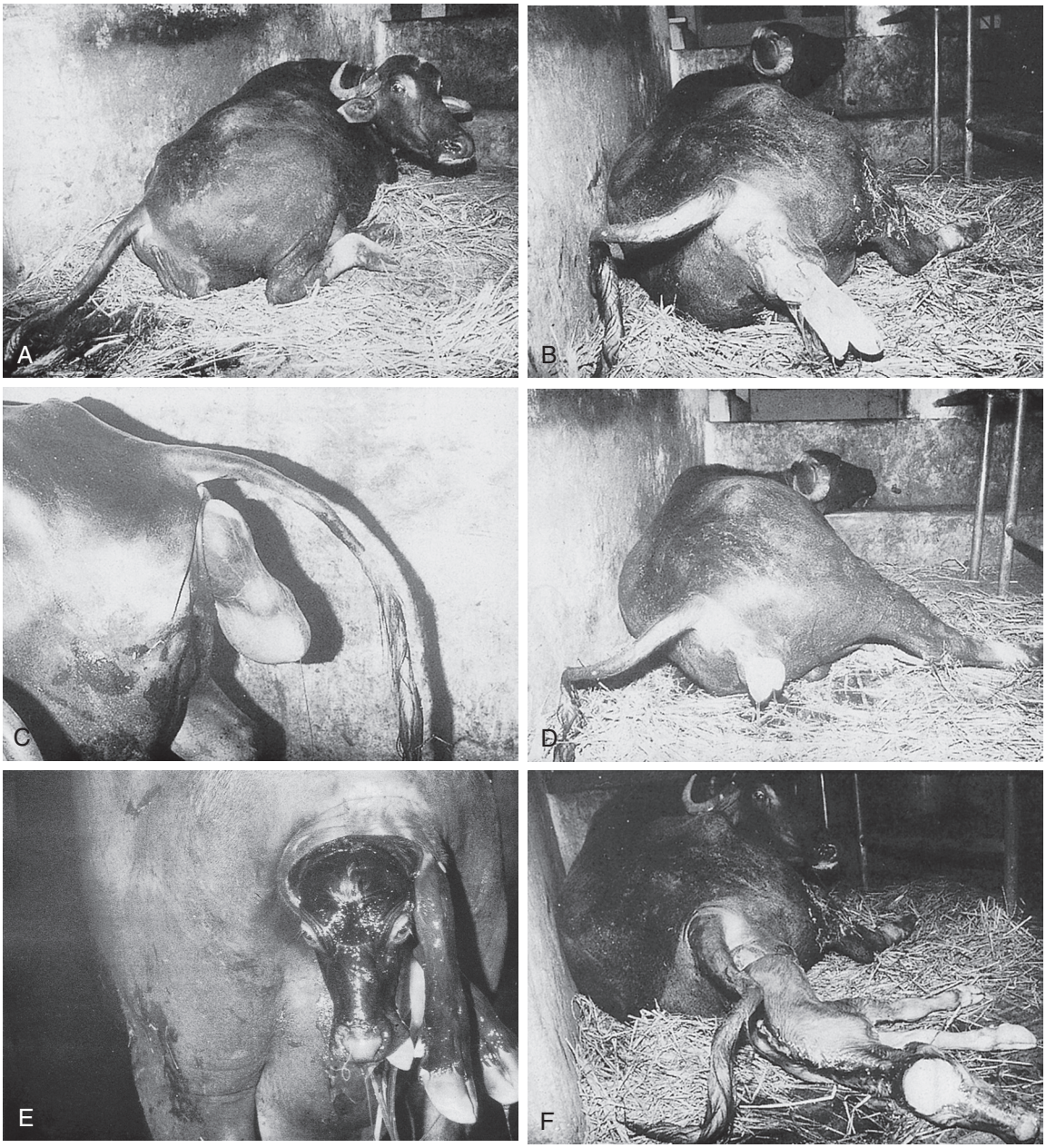

• **Fig. 38.3** Parturition in the buffalo. (A–E) Progression of second stage labour. (F) End of second stage labour.

in origin, relating to the relatively long uterine ligaments and low numbers of smooth muscle cells in the broad ligament of buffalo. The methods for correcting uterine torsion are the same as in cattle and include rotation of the fetus and uterus *per vaginum,* rolling of the animal using Schaffer's method, and laparohysterotomy (see Chapter 13).

Postpartum Disorders

The incidence of postpartum disorders is higher in the river than in the swamp buffalo. A review of reports on parturient and early postpartum disorders in the buffalo (Purohit & Markandeya 2015) shows incidences ranging from 6% to 59% for retained fetal membranes (RFM), 2% to 20% for genital prolapse, and 7% to 29% for metritis. Infections, uterine inertia, dystocia, and poor management practices have been implicated in the pathogenesis of RFM. Cervicovaginal prolapse occurs prepartum, whereas uterine prolapse occurs postpartum, usually within the first 6 hours after expulsion of the fetus and involves a complete eversion of the gravid uterine horn.

The Puerperium

Involution of the Uterus

The uterus undergoes a marked decrease in size during the first 2 weeks postpartum, and by Day 15 the gravid horn can be located at the pelvic brim, whereas the nongravid horn is in the pelvic cavity. The involution rate gradually decreases; from 21 to 25 days the rate of receding is considerably slow but progressive. The complete uterus returns to the pelvic cavity by Day 25, and the gravid horn attains normal tone and consistency by 30 to 35 days (Chauhan

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et al. 1977). The discharge of uterine lochia at first contains pure blood but changes thereafter to a chocolate colour and appears turbid due to a mixture of leucocytes and tissue debris. It increases up to about Day 7 postpartum, then decreases in volume and changes to a white mucoid discharge, which ceases within 2 to 3 weeks. Uterine involution is delayed in cases of calving problems such as dystocia, uterine prolapse, and RFM, and the discharge of lochia persists beyond the normal duration.

Resumption of Ovarian Activity

The CL of the previous pregnancy has completely regressed by Day 30 postpartum. Peripheral plasma progesterone concentrations decline rapidly after parturition to reach undetectable levels by Day 3 or 4 and remain so till the first postpartum ovulation, which occurs at about 60 days in the river buffalo and 96 days in the swamp buffalo (Jainudeen & Hafez 1993). Factors that delay resumption of cyclicity include: poor nutrition (Baruselli et al. 2001) leading to low body condition score (BCS); suckling management (Perera et al. 1987); and climate (Nanda et al. 2003) that also influences nutrition through feed quality and availability. Hand-milked river buffaloes have a lower incidence of postpartum anoestrus than suckled swamp buffaloes, and those calving during their normal calving season resume cyclical ovarian activity earlier than those calving in other seasons. A review of results from Egypt, India, and Pakistan (El-Wishy 2007) showed that only 34% to 49% of buffalo exhibited oestrus during the first 90 days after calving, and 31% to 42% remained anoestrous for more than 150 days. The first postpartum ovulation was frequently followed by one or more short oestrous cycles (< 18 days). The presence of buffalo bulls in a herd has a 'biostimulatory' effect, reducing cyclic irregularities and advancing the time of ovulation (Moioli et al. 1998). The management and treatment of postpartum anoestrus in the buffalo are discussed later in 'Female Infertility'.

Reproduction in the Male

Buffalo bulls used for breeding under farming systems that employ natural mating must be capable of efficiently detecting oestrus and serving a high proportion of cows. One bull can cover 40 to 50 cows per year, provided there is no marked seasonality in the occurrence of oestrus. In large herds requiring the use of several bulls, it may be necessary to introduce them in rotation because the presence of two or more bulls in the herd at the same time is likely to result in aggressive behaviour between them. When bulls are used as semen donors for AI, they must be of exceptionally high fertility, and the semen must be capable of withstanding the processing and storage procedures used.

Anatomy of the Reproductive Organs

The reproductive organs of buffalo bulls are similar to those of cattle bulls, but the testes and scrotum are smaller and the penile sheath is less pendulous. As in cattle, the testis and epididymis can be palpated through the scrotal wall, and the prostate gland, seminal vesicles, and ampullae of the ductus deferens can be palpated transrectally.

Puberty

The age and body weight at puberty in buffalo males is as variable as in females and is determined more by body weight than age. With adequate nutrition, the testis size increases slowly between 5 and 15 months, rapidly between 15 and 25 months, and again slowly between 25 and 38 months of age. Spermatogenic cell divisions commence at about 10 to 14 months of age, and active spermatogenesis can be seen by 14 to 16 months (Perera 1999). However, sexual maturation, as indicated by the presence of motile spermatozoa in the ejaculate, is attained between 24 to 30 months of age (Jainudeen & Hafez 1993). The faster growing F1 river and swamp crossbreeds reach puberty earlier than the slower growing swamp buffaloes. The concentration of testosterone is at basal level from birth to about 5 months of age, increases gradually up to 14 to 16 months, then increases more rapidly between 18 to 24 months, and reaches adult levels of around 3 nmol/l (~1 ng/mL) thereafter (Hemeida et al. 1985).

Seasonality and Endocrinology in Adults

Buffalo bulls are capable of breeding throughout the year, but some seasonal fluctuation in reproductive functions is evident in most countries. In India, semen volume, density, and initial sperm motility did not differ significantly between different seasons, but postfreezing motility varied significantly, being lowest during summer and highest during winter (Bahga & Khokar 1991). The concentration of LH and testosterone in the blood of buffalo bulls shows the typical episodic or pulsatile pattern of secretion that is seen in cattle and is influenced by seasonal factors (Agarwal et al. 1983).

Spermatogenesis

Among farm animals the buffalo has one of the shortest spermatogenic cycles. The durations of the seminiferous epithelial cycle and spermatogenesis are 8.6 and 38 days, respectively (Sharma & Gupta 1979). Spermatozoa of the buffalo are slightly smaller than those of cattle, and the head has a more rectangular shape. Their average measurements are: length of head 7.5 µm; width of head at widest point 4.9 µm; length of midpiece 11.7 µm; and length of tail 50 µm (Vale et al. 2014).

Examination of Semen

Semen is usually collected with a conventional bovine artificial vagina (AV); alternatively, bovine electroejaculators can also be used (see Chapter 35). Either a female or a castrated or intact male buffalo can be used as the teaser. The temperature of the water jacket of the AV should be about 42° to 46°C, and the pressure within the AV should be adjusted to suit individual bulls. Sperm concentration is increased by allowing two or three false mounts before the actual collection. The normal ejaculate collected with an AV is milky white to creamy in colour and varies from 1 to 6 mL in volume, with a sperm concentration of 500 to 1500 million cells/mL. The mass activity and individual motility of buffalo spermatozoa are lower than those in cattle. The parameters of semen quality are affected by precoital sexual excitement, number of false mounts, age, season of year, frequency of collection, diet, and fitness of the bull. The temperature/humidity index adversely affects the volume of semen produced, depresses sperm concentration and initial motility, and increases the production of dead and abnormal spermatozoa. The decline in serum thyroxine during the summer months depresses feed intake and metabolism and thus decreases sperm production. Similarly, increased body temperature has adverse effects on initial motility and the number of dead spermatozoa in the ejaculate. Bulls with obvious testicular asymmetry yield considerably fewer

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spermatozoa per ejaculate. The presence of pathogenical bacteria, e.g., *Pseudomonas* spp. and *E. coli,* can reduce sperm motility and increase the dead sperm percentage.

Artificial Insemination

In the Indian subcontinent, AI has been practised in the river buffalo for over 40 years but has lagged behind its use in cattle largely because of the difficulty of detecting oestrus. In addition, lower fertility rates obtained with chilled or frozen semen are constraints to the widespread use of AI in this species. Moioli et al. (1998) have shown that buffalo spermatozoa subjected to freezing and thawing have a shorter life span in the female genital tract than fresh semen. Buffalo semen differs from cattle semen in having higher concentrations of calcium, chloride, acid soluble phosphorus, and alkaline phosphatase, and in having lower concentrations of total electrolytes, protein, cholesterol, ascorbic acid, citric acid, and fructose (Vale et al. 2014). Because of these findings, various extenders different from those used in cattle have been developed for preserving buffalo semen in the chilled or frozen form; the results have varied. These include lactose–egg yolk–glycerol, lactose–fructose–egg yolk–glycerol, and Tris–egg yolk–glycerol. The use of more than 20% egg yolk does not enhance cryoprotection but can lessen sperm forward motility in the cervix as a result of increased viscosity. An equilibration period of 6 to 9 hours and glycerol concentrations of 5% to 7% are most frequently used. Extended semen is placed in 0.25 or 0.5 mL straws or paillettes, each containing 30 million spermatozoa. The straws are then exposed to nitrogen vapours at −120° to −140°C and stored in liquid nitrogen. Rapid thawing at 37° to 40°C for 10 to 20 seconds is preferred over slow thawing.

The postthaw progressive motility of buffalo semen varies from 35% to 60%. The timing of AI in buffalo follows the am/pm rule used in cattle (see Chapter 43). The optimum timing of AI is at the end of standing oestrus, somewhat later than in cattle (Moioli et al. 1998). One of the ways to determine this would be to assess when the cow refuses to stand to be mounted by the teaser bull. Because this is often not practicable in commercial herds, AI is usually performed 24 hours after the first signs of oestrus (Perera 1999).

As in cattle, separation of X and Y bearing sperms has been done using high-speed flow cytometric cell sorting (see Chapter 44). The use of such semen according to the 'low dose deep AI' technology, with 2.5 million sperm deposited near the uterotubal junction, has resulted in 43% conception rate with around 90% of fetuses corresponding to the predicted sex (Presicce et al. 2005).

Fertility and Infertility

Evaluation of Fertility

The overall reproductive efficiency of buffalo can vary widely between different farming systems. Animals kept under smallholder systems in some Asian countries are often subjected to harsh environments, with inadequate feed and low managerial inputs. They have poor fertility compared with those kept under improved production systems in regions with mild climates. Under optimum conditions, buffalo can achieve a first service conception rate (CR) of over 65% with natural service and of 40% to 50% with AI using frozen semen. In well-managed herds, overall annual pregnancy rates of over 80% can be achieved, with a calving interval (CI) of 14 to 15 months (Perera 2008). However, in many smallholder systems the annual pregnancy rates are only around 50% to 60%, with CIs of 18 to 24 months. In many situations, a buffalo produces, on average, two calves every 3 years.

Female Infertility

The main causes for infertility in buffalo females include delayed sexual maturity, prolonged postpartum anoestrus, repeat breeding, and abortion (see Chapter 22). The factors influencing these vary depending on the farming system and include climate, nutrition, management practices, and both specific and nonspecific infections.

Anoestrus

As in cattle, two forms of anoestrus occur in the buffalo. In the first form, the animal has small ovaries with no palpable CL and does not exhibit oestrous because she is acyclic (true anoestrus). In the second form, the animal possesses a palpable CL in one ovary but has not been detected in oestrus as a result of suboestrus or silent ovulation. A high incidence of true anoestrus occurs during the hot summer months and during periods of poor feed availability. Blood levels of calcium, phosphorus, glucose, and total proteins are lower in anoestrus than in cyclical buffalo. In most cases the disorder resolves spontaneously with the arrival of more favourable climatic conditions and adequate feeding. Restricting the suckling by calves and alleviating heat stress by wallowing or the use of water sprinklers may also help to initiate ovarian activity.

Hormonal treatment protocols for true anoestrus are similar to those developed for cattle (see Chapter 22). These include gonadotrophin-releasing hormone (GnRH) or its analogues, and progesterone or synthetic progestagens placed intravaginally or subcutaneously for 10 to 12 days, followed by equine chorionic gonadotrophin (eCG) or GnRH at the time of withdrawal (Baruselli et al. 2007). Variable results have been reported, with some successful trials showing induction of cyclicity in 81% of acyclic animals and with a conception rate of 74% at the end of the breeding period, compared with 54% for controls (Zicarelli et al. 1997). However, results were poor in some trials, particularly when other underlying causes of infertility, such as inhibitory effects of climate, management, nutrition, low BCS, and diseases had not been addressed before implementing hormonal therapy.

Suboestrus and silent ovulation were previously believed to be a major problem in buffalo breeding, but recent evidence suggests that it is due to the poor oestrous detection in these herds. The incidence has been reduced in herds in which improved oestrous detection practices have been implemented (Jainudeen & Hafez 1993). If the problem persists, hormonal treatments can be used for oestrous induction, thus removing the need for oestrous detection by using fixed time AI (see later under 'Improvement of Fertility').

Cystic Ovaries

The incidence of cystic ovaries is lower in buffalo than in cattle, and the condition is more common in the high-producing river type than in the suckled swamp type. In a survey, cystic ovaries accounted for 6% of reproductive failure in over 12,000 river buffalo in India; most cases occurred before Day 45 postpartum (Rao & Sreemannarayanan 1982). The clinical findings and treatment are similar to those in cattle (see Chapter 22).

Repeat Breeding, Endometritis, and Abortion

As in cattle, repeat breeding in buffalo could be due to either fertilisation failure or early embryonic death. The aetiologies may

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be related to disorders in either the female or male (including errors in AI), as well as to the environment and management. The incidence of repeat breeding in buffalo ranges from 8% to 32% (Rao & Sreemannarayanan 1982). It is lower in animals kept individually on smallholdings than in large herds, and in heifers compared with adult buffaloes up to the third parity; thereafter, the incidence decreases, probably because of culling of affected animals from the breeding stock.

Nonspecific uterine infections, leading to clinical or subclinical endometritis, are among the major causes of repeat breeding (Usmani et al. 2001). Factors attributed to the high incidence of endometritis in buffalo include unhygienic calving management, persistence of infection from the puerperal period, and midcycle inseminations. It can also be caused by the malpractice of stimulating milk let down through the introduction of instruments, sometimes the animal's own tail, or a hand into the vagina. Furthermore, because the vulvar labia are not closely opposed in the buffalo, wallowing in contaminated water may result in ascending infections. The common organisms isolated from cases of metritis and endometritis include *Arcanobacterium pyogenes; E. coli; Fusobacterium necrophorum;* and *Bacteroides* spp. (Azawi et al. 2008). The methods of treatment are the same as those for cattle (see Chapter 23). Other causes of repeat breeding include luteal dysfunction delayed ovulation or anovulation, and nutritional deficiencies, resulting in, for example, low serum calcium and phosphorus concentrations as well as hypoglycaemia. The factors related to AI include the quality of semen, timing of breeding, and technical skill of the inseminator.

The incidence of abortion in buffalo ranges from 1% to 6% and could be due to noninfectious or infectious causes. The specific infections that cause abortion in cattle, such as brucellosis, leptospirosis, campylobacteriosis, trichomoniasis, and infectious bovine rhinotracheitis (IBR), have a low incidence in the buffalo.

Male Infertility

The causes of infertility and sterility in buffalo bulls can be classified as either congenital or acquired. Congenital causes include structural defects in the testes (agenesis, aplasia, hypoplasia, or cryptorchidism), segmental aplasia or hypoplasia of the ductus deferens and epididymal ducts, or defects of the penis and prepuce (prolapse of prepuce, deviation of penis, or persistent frenulum). Such animals should not be used for breeding, as these disorders may be transmitted to the offspring, and they should be castrated or culled. Acquired causes of infertility include injuries and thermal stress, and specific as well as nonspecific infections that lead to inflammatory changes including orchitis, epididymitis, seminal vesiculitis, and testicular degeneration (Vale et al. 2008).

Another method of classification is based on the functional defect, as either *impotentia coeundi* (lack of sexual desire or libido and inability to copulate) or *impotentia generandi* (inability to fertilise due to poor semen production). The causes of reduced libido include age, poor nutrition, environmental stress, overuse, painful conditions, and hormonal disorders. Measures such as improved nutrition and housing, hoof care, exercise, and sexual stimulation can improve libido. Inability to copulate is caused by physical or functional defects that prevent mounting, erection, intromission, and ejaculation. The factors that lead to poor semen production are related to spermatogenesis, sperm maturation, or functions of the accessory glands.

Improving Fertility

The approach to improving fertility in buffalo includes the adoption of proven improved practices of management, including breeding, feeding, and disease control, which are suitable for the particular production system, and are cost effective using the available resources. Selective breeding among river breeds, crossbreeding between river and swamp breeds, and improvements in nutrition can advance the onset of puberty. Similarly, management practices such as early weaning, a high plane of feeding, and proper protection during the hot summer months can advance the restoration of postpartum cyclical ovarian activity, increase conception rates, and reduce the length of calving intervals. As in cattle, several assisted reproductive technologies (ARTs) have been applied in the buffalo, as described later in this chapter.

Oestrous Synchronisation and Oestrous Induction

The main objective of oestrous synchronisation (in a group of females) or oestrous induction (in individuals) is to overcome the problems in heat detection and enable fixed-time AI at a predetermined time. As in cattle, early studies were aimed at either inducing premature luteolysis of a CL, using $PGF_{2\alpha}$ or its analogues, or prolonging the luteal phase, using progesterone or progestagens (Perera 1987). However, later studies on the effect of follicular dynamics on the outcome of these procedures (Zicarelli et al. 1997) have led to methods aimed at manipulating follicular development to achieve better synchrony and improved fertility (Baruselli et al. 2007, De Rensis & Lopez-Gatius 2007). A review of results from various protocols for oestrous synchronisation (Borghese 2005) shows that CR achieved with prostaglandins, either alone or in combination with GnRH, ranged from 7% to 56%. CR using progesterone-releasing devices, either alone or in combination with eCG and in some cases further supplemented with human chorionic gonadotrophin (hCG) or GnRH, ranged from 8% to 64%. The 'Ovsynch' protocol (GnRH followed by prostaglandin 7 days later and a second GnRH 2 days later) has been used successfully in buffalo, with synchronisation of ovulation in 70% to 90% and CR of 33% to 60% (Paul & Prakash 2005). A third dose of GnRH given 6 days after insemination to induce accessory CLs has been reported to increase CR and birth rate (Carvalho et al. 2007; see also Chapters 8 and 25). In addition to the type of protocol selected, the following factors must also be addressed to achieve success in buffalo (Perera 2011): (a) selection of animals that are in good nutritional condition and free from disease; (b) prevention of stress during the procedures for treatment and AI, especially under tropical conditions when animals may be herded together, tethered, or moved to other locations; and (c) where seasonal differences exist, scheduling treatment for the more favourable periods or high breeding season when the majority of animals are likely to be cycling.

Difficulty of detecting oestrus in individual females can be overcome by oestrous induction to permit AI at a predetermined time. The two methods used are: (a) treatment with $PGF_{2\alpha}$ or a synthetic analogue to cause premature luteolysis; and (b) the creation of an artificial luteal phase by the use of a progesterone-releasing device for a period and its withdrawal. For the first method, it is necessary to confirm the presence of a CL and absence of pregnancy by rectal palpation before treatment.

Multiple Ovulation and Embryo Transfer

Although the first buffalo calf born after embryo transfer occurred in the United States in 1983 after the nonsurgical collection of

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a 7-day blastocyst and nonsurgical transfer to an unrelated river buffalo (Drost et al. 1983), the application of embryo transfer in buffaloes has lagged behind substantially that in *Bos taurus* (see Chapter 44). This has been due to several factors (Madan et al. 1996), including: (1) the poor superovulatory response using a variety of different protocols, attributed mainly to the smaller population of recruitable follicles in the ovary; and (2) a relatively low rate of transfer of oocytes to the oviduct and/or impaired transport of ova and embryos in the reproductive tract. In a large scale, multiple ovulation and embryo transfer (MOET) operation in India (Misra et al. 1994), the transfer of 469 embryos resulted in a pregnancy rate of 17% and a calving rate of 9.8%. In Italy, Campanile et al. (1995) transferred 76 buffalo embryos and obtained a pregnancy rate of 30%, with no significant difference between fresh or frozen-thawed embryos and between morulae or blastocysts. However, the overall rate of success in terms of calves born per superovulation is still too low for MOET to be widely applicable under practical farming conditions. Two situations in which it might have a role are for establishing nucleus breeding stocks or for conservation of genetic resources.

In vitro Embryo Production Including in vitro Maturation, Fertilisation and Culture

The sequence of procedures for *in vitro* embryo production (IVEP) involves recovery of oocytes from slaughtered animals by direct aspiration or from live animals by ultrasound-guided transvaginal ovum pick-up (OPU), followed by *in vitro* maturation (IVM), *in vitro* fertilisation (IVF), and *in vitro* culture (IVC). The OPU technique (see Chapter 44) can be used for repeatedly collecting oocytes in cycling buffalo once or twice every week or in prepubertal heifers and cyclic cows after treatment with FSH. The yield of cumulus–oocyte complexes (COC), using OPU in nonsuperovulated buffalo, is about 1 to 2 per ovary per collection, whereas after FSH treatment, it is about 2 to 3 per ovary. The cleavage rate of oocytes selected as being suitable for IVEP is around 50%, but only about 10% develop into morulae and blastocysts (Manik et al. 2006). Results from transfer of fresh embryos derived from repeated OPU in China show that a calving rate of 35% was obtained when recipients were normally cycling, compared with 15% in those subjected to synchronisation (Liang et al. 2007). Methods for cryopreservation of oocytes, using vitrification, are more successful than slow freezing, with ethylene glycol as the cryoprotectant. Transfer of vitrified embryos derived from oocytes collected from slaughtered river type animals resulted in a pregnancy rate of 16% and a calving rate of 10% to 11% in swamp and river type recipients (Hufana-Duran et al. 2007).

Cloning

Cloning by somatic cell nuclear transfer (SCNT) in buffalo is still in its early stages, but some basic understanding has been achieved regarding parthenogenetic, *in vitro,* and *in vivo* development of embryos reconstructed by transferring donor nuclei from fetal and adult fibroblast cells to enucleated buffalo oocytes. Embryos reconstructed using fetal fibroblasts were capable of developing to the blastocyst stage, and some pregnancies were detected after the transfer of cloned blastocysts into recipients, but none were carried to term (Saikhun et al. 2004). Cloned embryos that are capable of developing to the morula and blastocyst stages have also been constructed using enucleated rabbit oocytes as recipient cytoplasm and cow, swamp buffalo, pig, and elephant fibroblasts as donor nuclei (Numchaisrika et al. 2007). See also Chapter 44 for more information about cloning and embryo transfer.

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The cervids are a complex assemblage of taxa showing extreme diversity in morphology, physiology, ecology, and geographical distribution. Reproductive strategies adopted by various species are also diverse and include a range from highly seasonal to completely aseasonal birth patterns. The recent growth in knowledge of cervid reproduction is strongly biased towards the larger bodied, gregarious, mixed grazer-browser species that have adapted well to human management and commercialisation. These species tend to represent 'K-selected' climax species characterised by very productive annual breeding success, singleton births, and long breeding life (10 + years). Conversely, we know relatively little about the reproductive patterns of the 'r-selected' smaller bodied, solitary (and often highly territorial), forest-dwelling browser species, often characterised by great fecundity (twinning) and shorter breeding life (< 10 years). This group includes many of the endangered cervid taxa. This chapter describes and discusses the general reproductive biology and artificial reproductive technologies of cervids, as well as including more recent studies of cervid reproductive productivity. Male cervids are referred to as stags and females as hinds.

Distribution and Diversity of Cervids

Although the taxonomy of cervids is often debated (particularly at the species/subspecies level), Whitehead (1993) considers that deer are represented by 43 species and 206 subspecies distributed widely across Europe, Asia, North America, South America, and northern Africa. Their evolution and geographical radiation over the last 20 to 40 million years has resulted in remarkable diversity in morphology, physiology, and ecology. In particular, reproductive strategies among cervids vary widely within and between regions. As such, no one species can be considered to represent a 'typical' deer in terms of reproductive function. For example, some cervids exhibit highly seasonal patterns of birth in cool temperate climes, whereas others are aseasonal in equatorial regions. Also, although many species are strictly monovular and bear single offspring annually, others are normally polyovular and bear multiple offspring. Even embryonic development and placentation vary among species (Asher et al. 1999a,b). Harrington (1985) expressed this variation in terms of 'life-history strategies', in which cervids can be classified into the two extremes; one maximises the reproductive capacity of individuals and the potential growth rate of the population (r), and the other maximises the competitive ability of individuals and the stability of the population (K). Unstable or early successional habitats favour the r-strategy, whereas stable or late successional (climax) habitats favour the K-strategy (Harper et al. 1961). The r-strategists are identified by their greater reproductive potential, exhibiting multiple births, early sexual maturation (<1 year) and short breeding life (<10 years). They are predominantly territorial browser- or concentrate-feeders adapted to rapid colonisation of early stage successional habitats, including species such as roe deer (*Capreolus capreolus*), white-tailed deer (*Odocoileus virginianus*), moose (*Alcesalces*), and numerous species of tropical, forest-dwelling deer. K-strategists have lesser reproductive potential that includes lower fecundity (i.e., singleton births) and later sexual maturation (>1 year), and are generally mixed grazer/browsers with marked gregariousness and social/behavioural plasticity. They tend to occupy climax habitats and include nearly all taxa within *Cervus* (red deer, wapiti, sika deer, sambar deer, etc.) and *Rangifer* (reindeer, caribou). Because of their ability to adapt to human management regimens, it is the K-strategists that are predominantly represented in the research literature. It is therefore important to note that, although there was an increased understanding of reproductive function in cervids over the last few decades, this relates mainly to those species of importance to agriculture (i.e., deer farming) and a few endangered species undergoing ex situ conservation efforts. In general, the research has been largely directed towards successful application of assisted reproductive technologies to manipulate the genetic composition of populations (e.g., selection for improved agricultural productivity and avoidance of inbreeding depression) (see also Chapter 44). The studies to date are clearly biased towards a few key species, particularly the larger bodied, monovulatory species with greater environmental and behavioural plasticity (K-strategists) that makes them suitable candidates for captive propagation. The smaller bodied, forest-dwelling, and solitary species (r-strategists) are clearly underrepresented in such research. This later group includes numerous species whose populations are under threat or are endangered (see Asher et al. 1999a).

Seasonality versus Aseasonality

The necessity for most cervid species to give birth at an appropriate time of year for optimal survival and growth of offspring has exerted considerable influence on their reproductive physiology (Lincoln & Short 1980). Species of northern temperate or arctic origins typically conceive in autumn and calve in summer. In contrast, species of tropical origin often exhibit limited seasonality or are completely aseasonal (Lincoln 1985). The endogenous mechanisms governing seasonal reproductive patterns in temperate species are robust, being manifest rigorously when animals are transferred between localities despite subtle variations in seasonal feed supply. The transference of temperate species across the equator results in an exact 6-month phase change (Marshall 1937), even though the

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relationship between season and feed production differs considerably between continental northern hemisphere and insular southern hemisphere environments (Asher et al. 1993a). Tropical species transferred from equatorial zones exhibit either pronounced 'reverse seasonality' (spring conceptions and autumn/winter calving; e.g., rusa deer, *Cervus timorensis,* in southern Australia), wide birth seasons (e.g., chital deer, *Axis axis,* in Australia), or complete aseasonality (e.g., Reeve's muntjac, *Muntiacusreevsii,* in UK) (Chapman et al. 1984, Loudon & Curlewis 1988, Monfort et al. 1991, Mylrea 1992). It is noteworthy that there is a general paucity of information on the birth season of tropical species in their native tropical environment, although there are well-documented examples of species that exhibit strictly seasonal patterns of birth.

For temperate and arctic species it is generally accepted that entrainment of seasonal reproductive cycles is effected by endogenous recognition of photoperiod changes, with the majority of species initiating mating activity during the decreasing day length of late summer and autumn (Lincoln & Short 1980). Variations in the actual mating season between species of up to 8 weeks usually offsets differences in the duration of gestation such that parturition generally occurs in midsummer (Lincoln 1985). A notable exception among temperate species is the Père David's deer (*Elaphurus davidianus*), which initiates mating activity in early summer and calves in late spring, despite an unusually long gestation of approximately 280 days (Wemmer et al. 1989).

There is increasing evidence that some tropical species also perceive and respond to changes in photoperiod, although in a different manner than temperate species. Rusa deer living in the tropics exhibit asynchronous antler cycles and birth timings. However, once translocated to temperate, high latitude zones, they appear to establish an evolutionary-based, 'long-day' reproductive rhythm (Van Mourik & Stelmasiak 1990). Brow-antlered Eld's deer (*Cervus eldithamin*) exhibit strong reproductive seasonality in their subtropical native range. This pattern persists after translocation to high latitudes (48°N), even though this strategy results in the birth of offspring during harsh winter months (Monfort et al. 1991). It is hypothesised that Eld's deer respond to the relatively subtle, low amplitude, photoperiodic oscillations experienced in their native subtropical latitudes. Higher latitudes simply serve to reinforce or strengthen seasonal rhythms because the direction of photoperiodic change is similar but of greater amplitude. Chital deer appear to exhibit only limited seasonality within their native tropics and after translocation to temperate zones. However, exogenous melatonin treatment has been shown to hasten and synchronise antler casting among stags (Mylrea 1992), indicating possible photoperiodic responsiveness in a species that, in other respects, appears unresponsive. In contrast, tropical sambar deer (*Cervus unicolor*) acclimatised to temperate zones in New Zealand exhibit a loose pattern of 'reverse seasonality' in calving but fail to show any discernible seasonal patterns in prolactin secretion (Asher et al. 1997a).

Because tropical cervid species are not exposed to strong circannual photoperiodic rhythms, reproductive patterns may have evolved in response to a variety of factors, including seasonal fluctuations in the availability of specific food items, local resource competition among sympatric species (species that occupy about the same area of land but do not interbreed), or predation pressure; many of these factors may be directly related to rainfall patterns. For example, it has been asserted that conceptions are timed in white-tailed deer living in Colombia (5°N) and the Everglades National Park (25°N) so that births occur during the dry season (Blouch 1987, Smith et al. 1996). The fawning season of hog deer (*Axis porcinus*) in Nepal (27°N) coincides with new vegetative growth that occurs after seasonal grass fires caused by lightning strikes or agricultural burning; it is believed to have been occurring for thousands of years (Mishra & Wemmer 1987). However, how these species synchronise their season of conceptions to achieve birth synchrony remains to be resolved. Even species such as sambar deer and chital deer, sharing the same habitat in Nepal, fawn 6 months out-of-phase to one another (Mishra & Wemmer 1987).

Cervid species occupying wide latitudinal and longitudinal ranges have generally developed discrete populations that exhibit pronounced phenotypic and genotypic differences. For example, the red deer/wapiti species *(Cervus elaphus)* includes at least 23 recognised subspecies in a west/east cline, from Western Europe to North America (Whitehead 1993). Climatic variation between subspecies zones may be associated with subtle variation in reproductive seasonality of the various subspecies. Studies have indicated that Western European (Scottish) red deer (*C.e. scoticus*) and Eastern European red deer (*C.e. hippelaphus*) farmed in New Zealand exhibit a 2- to 3-week difference in rutting activity and subsequent mean calving dates (Scott et al. 2006). These differences occur at the same latitudes in both Europe and New Zealand (and indeed within sympatric populations on New Zealand farms), indicating subtle genetic differences in subspecies responses to the prevailing photoperiodic regimen.

Nonpregnant Female

With few exceptions, female cervids (hinds) are polyoestrous, with nonpregnant animals exhibiting continuously repeated oestrous cycles (some tropical species) or, more commonly, alternating periods of oestrous cyclicity and anoestrus. Oestrous cycles have been characterised for a number of species based on studies of oestrous behaviour and/or luteal secretion of progesterone. In nonpregnant females of temperate species, such as red deer and fallow deer, the onset and termination of oestrous cyclicity occur in autumn and spring respectively, with 5 to 8 cycles expressed. Anoestrus is characterised by low peripheral plasma concentrations of progesterone indicative of complete ovulatory arrest and may persist for 4 to 6 months from spring to early autumn. In marked contrast, some tropical species, such as brow-antlered Eld's deer and rusa deer, translocated to higher, temperate latitudes, exhibit 'reverse seasonality', initiating oestrous cycles in spring and entering anoestrus in the following autumn. This 6-month phase shift in tropical species within temperate climes possibly indicates retention of photoperiodism from a temperate ancestral form. Some tropical species, such as chital deer, appear to have become completely aseasonal, with females exhibiting recurring oestrous cycles in the absence of pregnancy (Mylrea 1992). There are indications for some tropical species within temperate latitudes, such as sambar in New Zealand, that responsiveness to photoperiod has been lost, as indicated by an inability to detect measurable levels of plasma prolactin in sambar hinds during the year (Asher et al. 1997a).

The transition into the female breeding season is characterised by 'silent ovulations' (ovulations not preceded by overt oestrus) and short-lived (8–10 days) corpora lutea in most cervid species studied. In fallow deer (*Damadama*), multiple successive silent ovulations leading up to the start of the breeding season have been observed (Asher 1985). The transient nature of the preliminary corpora lutea may actually serve to promote within-herd synchrony of first overt oestrus of the season.

Subsequent oestrous cycles are generally of normal duration, as determined genetically for each species, although occasional 'long

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cycles' have been observed in some Père David's deer and Eld's deer (Curlewis et al. 1988, Monfort et al. 1991). Average duration of the normal oestrous cycle ranges from 17 days in sambar and axis deer, 18 to 20 days in red deer and Eld's deer, 21 to 23 days in fallow deer, and 24 to 27 days in moose and black-tailed deer *(Odocoileus hemionus)*. The mean duration of the oestrous cycle tends to increase progressively during the breeding season in most of these species (Guinness et al. 1971, Asher 1985, Curlewis et al. 1988, Monfort et al. 1991, Chapple et al. 1993, Schwartz & Hundertmark 1993, Wong & Parker 1993).

Overt oestrous behaviour has been observed in a number of cervid species, but the duration of mating receptivity at a single oestrus has been difficult to determine. In some species at least, including red deer and fallow deer, overt oestrus is generally terminated after successful copulation and may only persist for a matter of minutes. In the absence of copulation, oestrous behaviour in these species has been observed to occur for up to 24 hours (Asher et al. 1993b). Early studies on both of these species have shown that the onset of oestrus coincides with the onset of the preovulatory LH surge, which itself peaks about 24 hours before ovulation (Berg et al. 1994, Asher et al. 1999a).

Luteal events during the cervid oestrous cycle are similar between species. Luteinisation of postovulatory follicles is associated with increased secretion of progesterone, with maximal concentrations in peripheral blood occurring between days 10 and 16 of the oestrous cycle (Day 0 = oestrus). Although absolute plasma concentrations vary between species, the relative changes from Day 0 are quite similar between species (Asher et al. 1999a). Luteal regression, which for fallow deer involves the classic interaction between oxytocin and prostaglandin $F_{2\alpha}$ (Asher et al. 1988a), occurs rapidly about 1 to 3 days before the return to basal progesterone concentrations and return oestrus.

Ovarian follicular dynamics during the oestrous cycle have been described from ultrasonographic studies on red deer, fallow deer, and, more recently, North American Wapiti (C.e. manitobensis) (Asher et al. 1997b, 1999b, McCorkell et al. 2006). These studies, all of which involve monovulatory species, clearly demonstrated discrete, nonrandom patterns of antral follicular growth and regression during the oestrous cycle, with individual cycles characterised by two to four dominant follicle waves similar to that observed in cattle.

Female puberty, defined as the age of first ovulation, normally occurs between 1 and 3 years of age in most cervid species, although this is strongly influenced by climatic and nutritional factors. Such influences are mediated through body mass, as demonstrated for red deer hinds (Hamilton & Blaxter 1980, Clutton-Brock et al. 1982). Red deer hinds on New Zealand deer farms are expected to attain puberty at 16 months of age and produce their first calf at 24 months of age. However, high incidences of reproductive failure within populations of rising 2-year-old hinds (i.e., 10%–60% of hinds not attaining pregnancy) have been common (Asher & Pearse 2002), prompting research into factors controlling entry into puberty. Studies involving observations of over 20,000 young hinds have clearly shown a strong relationship between live weight at 16 months of age and the ability to enter puberty (Asher & Cox 2013), with hinds that fail to attain about 70% of the expected mature body mass of their genotype (subspecies) by 16 months of age, thus delaying puberty by at least 1 year. This has been termed as the 'permissive body mass threshold for entry into puberty' (Asher & Cox 2013). However, recent studies have provided support for the hypothesis that the nutritional environment in early life (birth to 3 months of age) influences the permissive body mass threshold for puberty at 16 months of age. Detailed analysis of a multiyear data set, in which the relationship between live weight and predicted pregnancy rate was modelled, demonstrated that hinds under nutritional constraints early in life had a higher permissive body mass threshold for puberty for their genotype, thus raising intriguing possibilities self-regulatory mechanisms to control reproductive processes in the face of variable environmental (e.g., climatic and nutritional) conditions (Asher & Cox 2013).

Pregnant Hind

Relatively little research has been dedicated to pregnancy establishment and maintenance in cervids. In the seasonally breeding red deer hind, conception rates after natural mating during the autumn rut appear to be high (>85% per mated oestrus), and early embryonic mortality (i.e., within 20 days of fertilisation) appears to be low (i.e., <1%; Berg et al. 1994). Given that hinds are polyoestrus, under circumstances in which nutrition and disease are not limiting, adult female red deer generally exhibit greater annual fertility (i.e., >96% pregnancy rates), albeit with lesser (i.e., <1% of pregnancies) twinning (Asher & Pearse 2002). This overall pattern of greater annual fertility appears to be a common feature of many cervids, although actual fecundity varies between species. As expected for the greater fecund species, such as white-tailed deer and roe deer, the actual fecundity (i.e., incidence of multiple fetus gestations) in a given year and locality is strongly influenced by habitat and nutritional quality variables.

Oocyte maturation, fertilisation, and early embryonic development have recently been studied *in vivo* and *in vitro* for red deer (Berg et al. 2008). These studies have shown that these events in red deer are similar to those occurring in other domestic ruminants, with the exception that the red deer embryo enters the uterus 2 to 3 days later at the blastocyst stage, 5 to 6.5 days after ovulation. Embryonic implantation in red deer occurs shortly before Day 27 after ovulation (McMahon et al. 1997).

Luteal support of pregnancy in red deer persists through the term of gestation, with peripheral blood progesterone concentrations remaining elevated to within 1 to 2 days of parturition. The contributory role of placental progesterone production in pregnancy maintenance is unknown. Studies on red deer and white-tailed deer clearly show that the presence of a functional corpus luteum is a requisite for a viable pregnancy (Asher et al. 1996, Plotka et al. 1982). However, ovariectomy of reindeer in late pregnancy was not always associated with abortion, indicating a possible contributing role of the fetoplacental unit in progesterone secretion and pregnancy maintenance in this species (Sjaastad et al. 1990).

Cervid species have gestation lengths ranging from 200 to 280 days, loosely correlated with species mature body mass. There is a general consideration that gestation length for any given species is genetically programmed and relatively robust in the face of environmental variables. Recognition is given to the influence of fetal genotype as being an important determination of gestation length (Kenneth & Ritchie 1953, Racey 1981). This is observed particularly with crossbreeding of cervid subspecies, for which there are considerable differences in their respective gestation lengths. For example, Western European red deer (*C.e. scoticus*) have an average mating–birthing interval of 233 days (Guinness et al. 1971) and that for North American wapiti (*C.e. manitobensis*) is 247 days (Haigh 2001). However, red deer hinds gestating F1 crossbred red × wapiti fetuses show an average mating–birthing interval of 239 days (Asher et al. 2005b,c). An even more extreme effect of

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fetal genotype is seen in hybridisation of red deer and Père David's deer (Asher et al. 1988b).

However, it has become apparent that gestation length within red deer subspecies is much more variable than previously thought (Asher 2007). Asher et al. (2005a) observed a marked variation in gestation length between hinds that were fed either greater or lesser amounts of nutrients during the third trimester of pregnancy. In that study, gestation length was negatively correlated with change in hind live weight, and the authors considered that under conditions of modest feed imbalance that appeared to influence fetal growth trajectory, a gestation length compensatory mechanism ensured optimal birthweight. In effect, the hypothesis argued that gestation length matched fetal growth rate to ensure optimum birthweight (possibly at the expense of optimal birth date).

Other research on red deer has demonstrated a statistically robust association between conception date and gestation length for red deer subspecies. Garcia et al. (2006) observed that Spanish red deer hinds (*C.e. hispanicus*) that were artificially induced to conceive up to 68 days before the standard mating date exhibited longer gestation lengths than those conceiving later in the natural breeding season. They postulated that hinds were able to modify gestation length to counteract out-of-season breeding to optimise calving date. However, these previous data were confounded by the fact that early conceiving hinds exhibited lower live weight gains over pregnancy, thereby adding some support to the hypothesis of Asher et al. (2005a) that nutrition was a driver of gestation length variation. Most recently, Scott et al. (2008) investigated the relationship between conception date and gestation length in Scottish and Eastern European subspecies of red deer (*C.e. scoticus* and *C.e. hippelaphus*). Their data supported a hypothesis that within-herd synchrony of red deer births is facilitated by a 'push/pull' control over gestation length such that hinds conceiving early and late in the breeding season have longer and shorter gestation periods, respectively. The individual data sets indicate that, for every 10 days difference in conception date, there was a change in gestation length of 1.9 to 4.9 days. Recent studies by Scott et al. (2015) provide support for the hypothesis that variation in fetal age during the latter stages of pregnancy, when feed quality and voluntary feed intake cycles of dams are in a state of flux, drives differential growth trajectories for early and late conceiving fetuses, leading to nutritional control over fetal maturation and initiation of parturition.

The aforementioned studies on red deer subspecies indicate a hitherto unexpected environmental control over gestation length in at least some cervids. Recent information indicates that this may also be the case for reindeer (Rowell & Shipka 2009) and sika deer *(Cervus nippon)* (Matsuura et al. 2004).

It is important to note that these studies so far cited may not be representative of cervids in general. Placentation systems vary across cervid species (McMahon et al. 1997), and in the case of one species, the roe deer, a form of embryonic diapause/delayed implantation occurs to lengthen the interval between mating and births in order to ensure optimal birth seasonality (Short & Hay 1966). This is the only ruminant species known to exhibit delayed implantation.

Ultrasonographic Pregnancy Diagnosis

Pregnancy diagnosis in deer has largely relied on real-time B mode ultrasonography to visualise the postimplantation fetus after about 25 to 30 days post-conception/mating. Again the development of this technology for use in deer has been mainly for application in the reproductive management of farmed red deer (White et al. 1989) and fallow deer (Asher et al. 1992b). Ultrasonography has more recently been refined for fetal ageing in red deer in order to estimate conception date and, hence, predict calving date (Revol & Wilson 1991) and for real-time assessment of ovarian follicle dynamics during anoestrus and the oestrous cycle in red deer (Asher et al. 1997b), wapiti (McCorkell et al. 2006, 2007), and fallow deer (Asher et al. 1999).

Reproduction in the Stag

Circannual variation in testicular function is evident in most deer species, reflecting changes in fertility, gross morphometry, and behaviour. Of the temperate species, these changes have been best described for red deer and fallow deer (see Asher et al. 1999). Episodic and pulsatile secretion of LH and testosterone change dramatically through the seasons, being maximal during 'rutting' (hypersexual activity by males around the short mating period in autumn). Similar patterns of secretion have been observed for Eld's deer, a tropical but seasonally breeding species (Monfort et al. 1993).

Testosterone-mediated changes in secondary sexual characteristics are very pronounced in both temperate and tropical cervid species. The antler cycle is linked closely to the testis cycle, with antlers cast annually when testes regress to their minimal dimensions. Casting is in response to a marked decline in testosterone secretion and can be induced by castration (Goss 1983). New antler growth occurs during the quiescent phase of the testes in the relative absence of testosterone secretion. Mineralisation (calcium phosphate deposition) of antlers coincides with increasing testicular activity and increasing testosterone secretion, and hard antlers are generally retained when testicular tissue is actively secreting moderate to high amounts of testosterone. This relationship holds for most antlered species studied (Goss 1983), although the seasonal patterns vary between species and the antler–testis cycles may not be synchronised among individuals of some tropical species (Loudon & Curlewis 1988).

Circannual patterns of testicular regression and recrudescence occurring in temperate cervid species relate to annual cycles of sperm production. Unlike most seasonally breeding domestic ruminants, which show moderate fluctuations in testicular activity (e.g., sheep), temperate cervids exhibit alternating periods of sperm production and complete spermatogenic arrest that reflect about fivefold changes in seasonal testicular volume (Lincoln 1971). Although tropical cervid species often exhibit circannual cycles of reproductive development, either in a synchronous (e.g., Eld's deer) or nonsynchronous (e.g., chital deer) manner, males appear to retain a degree of fertility throughout the year (Loudon & Curlewis 1988, Chapman & Harris 1991, Mylrea 1992, Monfort et al. 1993).

Assisted Reproductive Technologies

The application of artificial reproductive technologies to deer largely relates to genetic management of captive populations, either as farmed livestock (e.g., genetic selection for improved productivity) or as captive zoo populations of endangered species (e.g., *ex situ* management to prevent inbreeding). The developments of these technologies for deer have mostly stemmed from their application to the farming of red deer and fallow deer in New Zealand (and increasingly adopted overseas for the farming of these species), but more recently the techniques have been tested and modified,

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with variable degrees of success, for application in *ex situ* wildlife management. See also Chapter 44.

Artificial Insemination

Artificial insemination (AI) maximises the genetic influence of elite males by disseminating male gametes (spermatozoa) across a larger female base than is possible by natural mating systems. It also allows for individual sires to be used across multiple populations at the same time. This is fundamental to the application of genetic improvement programmes for livestock. Presently in the New Zealand deer farming industry, which has a national deer recording and genetic evaluation scheme (DEERSelect), between 20,000 and 30,000 red deer hinds are artificially inseminated each year, representing between 4% and 5% of the national breeding herd. The components of AI, namely semen collection/cryopreservation, oestrous synchronisation, and insemination, were developed for farmed red deer and fallow deer during the 1980s and early 1990s; however, the fallow deer industry did not gain momentum, and there is presently little adoption of AI in this species. The developments of AI techniques for red deer have been reviewed by Asher et al. (2000), Rhodes et al. (2003), and McMillan and Asher (2007), and given that AI on commercial red deer farms routinely results in average conception rates of between 65% to 75%, there have been few incentives to further improve techniques for this species (McMillan & Asher 2007). AI has also been used to produce F1 hybrid progeny between red deer and Père David's deer (Asher et al. 1988b) and between red deer and sambar deer (Muir et al. 1997).

Semen collection from male red deer is invariably done by electroejaculation after general anaesthesia or light sedation. The highly aggressive nature of reproductively-active red deer stags precludes more natural methods of collection (e.g., artificial vagina) that require the close presence of people. Ejaculates generally contain very high concentrations of spermatozoa ($1–3 \times 10^9$/mL) during the autumn/winter breeding season, but stags are effectively infertile outside of this period. Viable semen can be collected from rising 2-year-old (pubertal) stags, but generally semen volumes and spermatozoa densities increase with increasing age. Thus, although commercial operators have been able to obtain 25 to 49 straws of semen (i.e., individual AI doses) per ejaculate from younger deer, older stags generally yield 55 to 240 straws per ejaculate (McMillan & Asher 2007). Semen straws (0.25 mL volume) are generally loaded at between 5×10^6 and 20×10^6 viable (live) spermatozoa each for cryopreservation but may be loaded down to as few as 1×10^6 viable spermatozoa for fresh use within 24 to 48 hours of collection. Various citrate-based or Tris-based diluents and various cryoprotectants have been evaluated for red deer and fallow deer semen (Asher et al. 2000). All have proven to be effective for these species.

Synchronisation of oestrus in deer has been generally adopted as a more efficient and cost-effective alternative to detection of natural oestrus. As with other domestic ruminants, synchronisation can be achieved by either simulating the activity of the corpus luteum, through administration of progestogens, or by shortening the luteal phase of the oestrous cycle by administration of a luteolysin (prostaglandins). Oestrous synchronisation protocols for red deer have been described in detail by Asher et al. (2000). The vast majority of artificial inseminations in New Zealand (and probably other countries) use the CIDR device containing progesterone. Intravaginal insertion of CIDR devices for periods of between 12 and 14 days during the breeding season is generally effective in promoting a synchronised oestrus within 48 hours of device removal in red deer (Asher et al. 1992a). This regimen has also proven successful in fallow deer, chital deer, Eld's deer, and sambar deer (see Asher et al. 2000). However, the additional administration of equine chorionic gonadotrophin (eCG) at or near the CIDR device withdrawal is generally performed for red deer, particularly if synchronisatin is attempted close to the onset of the breeding season (Fennessy et al. 1990, 1991). Interestingly, the use of eCG is contraindicated in fallow deer due to the increased incidence of both anovulation and multiple ovulation as well as fertilisation failure (Jabbour et al. 1993). The ability of prostaglandins to synchronise oestrus is dependent on the presence of an active corpus luteum at the time of treatment, which limits synchronisation programmes to a period after the onset of natural ovulatory activity. Also, the red deer corpus luteum has been shown to be responsive to exogenous prostaglandin only during the latter half of the oestrous cycle, with the transition from refractoriness to responsiveness ranging from Day 6 (Asher et al. 1995a) to Day 11 (Fisher et al. 1994). Although oestrous synchronisation after injections of prostaglandin analogues has been reported for red deer, wapiti, fallow deer, Père David's deer, white-tailed deer, and reindeer (see Asher et al. 2000), the fertility of prostaglandin-induced ovulation has not been widely evaluated.

Insemination procedures for deer are either laparoscopic intrauterine insemination for smaller bodied deer of less than 100 kg body mass (e.g., fallow deer; Asher et al. 1992b) or transcervical intrauterine insemination for larger bodied deer of greater than 100 kg body mass (e.g., red deer; Rhodes et al. 2003). Whereas laparoscopic procedures invariably require general anaesthesia, transcervical inseminations, involving rectal manipulation of the reproductive tract, are generally performed without any form of chemical restraint but requiring physical restraint.

Multiple Ovulation Embryo Transfer

Multiple ovulation embryo transfer (MOET) is well-established as an applied tool for genetic improvement of agriculturally important livestock species and has the potential to assist in the genetic management of some endangered mammalian species (Wildt et al. 1992). (See also Chapter 44.) The development and application of MOET technology to cervid species has occurred sporadically over the last 30 years, with studies focussing on red deer, wapiti, fallow deer, and white-tailed deer (see Asher et al. 1995b). Success rates, in terms of multiple ovulation (superovulation), fertilisation, embryo recovery, and surrogate pregnancies, have been highly variable between studies, and for some species, results have often been disappointing (Fennessy et al. 1994). Although superovulation and embryo recovery/transfer procedures do not appear to have radically changed from those described for red deer by Fennessy et al. (1989, 1994), outcomes of continuing MOET programmes on farmed red deer in New Zealand have become more consistent and economically viable, and many stud farms run annual MOET programmes to capture more rapid genetic gains from female lineages. Publications outlining such outcomes are sparse due to commercial sensitivities. However, it is often stated by commercial operators that the biggest factor influencing outcomes is donor hind stress. Well-habituated (tame) hinds tend to produce more viable ovulations/fertilised embryos than hinds of a nervous disposition.

Superovulation programming of donor red deer hinds is similar to that employed for domestic ruminants, generally involving intravaginal progestogen synchronisation (more often than not, CIDR devices) alongside stimulation of follicle development with

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exogenous gonadotrophin (usually FSH but also may additionally include eCG) towards the final stages of progestogen treatment (Fennessy et al. 1994). Subsequent procedures for embryo recovery, recipient synchronisation, embryo transfer, and embryo cryopreservation differ little from other ruminant species and are described by Fennessy et al. (1994). Average success rates for farmed red deer in terms of viable recipient pregnancies per donor programmed are reputedly between 3 and 8 scanned pregnancies. For some donor individual hinds, the success rate has exceeded 10 to 12 surrogate pregnancies.

IVF and Cloning

The *in vitro* embryo production system developed for farmed red deer has evolved along similar lines to that pioneered for other domestic animal species and was the platform for further advanced reproductive technologies such as cloning (Berg & Asher 2003). However, applying existing *in vitro* embryo production methods from other species resulted in limited success (Fukui et al. 1991) and necessitated the development of a species-specific methodology for red deer based on their culture media system, Deer Synthetic Oviduct Fluid (DSOF); this resulted in the successful culture of red deer embryos to the blastocyst stage (Berg et al. 1995a, 2002a, 2002b) and ultimately led to the successful production of recipient pregnancies and the live birth of calves (Berg et al. 1995b, 2002c). IVF systems have also been applied, with varying degrees of success, to wapiti, sika deer, axis deer, and reindeer (see Berg & Asher 2003) but have yet to gain any significant traction in genetic management of farmed deer and captive populations of endangered deer due to overall low efficiency at various points along the pathway (i.e., oocytes recovered, oocytes fertilised, viable pregnancies, live births). Recently, red deer were cloned by somatic cell nuclear transfer (SCNT), using multipotent antler stem cells and their differentiated progeny (Berg et al. 2007). The efficiency of the system was again low throughout the process. Of 70 SCNT embryos implanted into recipient hinds, there were 22 viable pregnancies scanned at Day 35, but only 11 developed to term, of which 8 survived to weaning at 3 months of age (Berg et al. 2007). Of significance, however, the surviving clones remained healthy into adulthood.

Reproductive Productivity of Farmed Red Deer

Cervid species generally adhere to an annual reproductive cycle, whether it be seasonally delineated or aseasonal. As with all annual breeders, success or failure to annually rear an offspring (and in some cases, more than one offspring) is often driven by environmental variables that influence any one of a number of points along the reproductive cycle. Innumerable studies on free-ranging cervid populations have demonstrated causes of lost reproductive potential, ranging from nutritional effects on ovulatory success to predation effects on neonate survival. It is not the purpose of this chapter to delve into extrinsic factors influencing annual reproductive productivity of wild deer populations, as these are generally outside the scope of veterinary and husbandry practitioners. However, the reproductive management of farmed deer and captive zoo populations of deer do fall under their auspices. By far, the most important issues pertaining to reproductive management of managed deer herds are nutrition and infectious disease (and in many cases these are linked together).

Using farmed red deer in the New Zealand pastoral environment as an example, reproductive management aims to maximise annual breeding outputs by eliminating or minimising sources of reproductive wastage. Annual reproductive productivity is still a major driver of on-farm profitability and, at its simplest and most realistic, is expressed as the number of surviving offspring produced annually per breeding female (often termed 'weaning rate' in New Zealand). However, this metric hides various forms of reproductive wastage. Key areas of focus are hind fertility (ovulation), fertilisation (mating management), fetal mortality (resorption/abortion), and neonate mortality (Asher & Pearse 2002).

Infertility of Hinds

Failure of hinds to attain reproductive condition each year is essentially the failure to initiate seasonal ovulatory cycles. Although this has often been thought to be the underlying cause of low reproductive performance in farmed red deer, anovulation in adult (>2 years old) hinds appears to seldom account for more than 1% to 2% of overall wastage. Exceptions to this on individual farms in some years clearly relates to extremely harsh environmental conditions before the mating season (particularly over summer lactation) that severely compromise body condition (depleted body fat and muscle catabolism), leading to anovulation within the adjacent breeding season (Asher & Pearse 2002). This phenomenon has been very well demonstrated for wild red deer occupying marginal habitats on the Isle of Rhum, Scotland, in which hinds that invest heavily in their calf's growth during summer can fail to become pregnant in the following breeding season (Guinness et al. 1978; Clutton-Brock et al. 1982). However, these are cases of temporary cessation of ovulatory activity driven by negative energy budgets before the breeding season, and affected hinds may simply skip a single breeding season to reinstate reproductive processes in subsequent years. Studies on farmed red deer by Pollard et al. (2002) showed that modest differences in body condition score (BCS) between hinds before and during the breeding season strongly influenced conception date, with hinds of lower BCS conceiving 8 to 12 days later in the season than hinds of the highest BCS. However, this study did not investigate the lower boundaries of body condition that could lead to seasonal anovulation.

Young hinds entering their first breeding season (post-pubertal) at 16 months of age are more likely to exhibit anovulation than adult hinds. Failure of red deer hinds to attain puberty at 16 months of age has been widely reported around the world and invariably relates to poor nutrition that has compromised prepubertal growth. In such cases, puberty is delayed until successive breeding seasons (Hamilton & Blaxter 1980). The relationship between body mass (live weight) and puberty incidence was investigated in detail for a range of red deer genotypes (subspecies and their crossbreeds) of differing mature body mass by Asher and Cox (2013). Analysis of live weight and pregnancy diagnosis records of more than 6000 young hinds across several New Zealand deer farms demonstrated the profound relationship between premating season body mass (i.e., live weight at 14–16 months of age) and pregnancy success/failure, thus indicating that hinds must attain a minimum of 65% to 70% of their genotype's expected mature hind body mass by 16 months of age to enter puberty. This has been termed the 'permissive threshold body mass' for entry into a pubertal state (i.e., body mass at which there is a 50% probability of hinds attaining puberty). However, the study demonstrated that early life growth (i.e., between birth and 3 months of age) influenced the permissive body mass threshold for any given genotype,

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such that the threshold increased for hinds that had experienced growth retardation during this period. The authors postulated that early life somatic growth influences the rates at which different tissues are accreted within the body leading up to the first breeding period, such that hinds of similar body mass may vary in the ratio of muscle to fat. Thus, whereas body mass has in the past been viewed as the key determinant of permissiveness to enter a pubertal state for female red deer, it may be that it is actually components of body tissues that are the important determinants of puberty attainment (Asher & Cox 2013). This hypothesis has yet to be tested for red deer.

Embryonic Death and Abortion

Although previously considered of low importance to overall reproductive wastage in farmed red deer in New Zealand (Asher & Pearse 2002), fetal wastage has more recently received considerable attention after the demonstration by B-mode ultrasonography of fetal disappearance between early and late pregnancy (Wilson et al. 2012). Subsequent studies have demonstrated incidences of fetal loss ranging from 0% to 15% across New Zealand deer farms, with the incidences for first pregnancy (pubertal) hinds being approximately twice that of older hinds, with the overall incidence across monitored farms being approximately 2% (Patel 2016). Epidemiological studies have failed to identify a single causative factor, although there is compelling evidence implicating acute *Toxoplasma gondii* infection on some affected farms (Patel 2016).

Perinatal Calf Mortality

Perinatal calf mortality is a significant cause of lost reproductive potential on New Zealand deer farms. Although the causation is highly variable, a number of studies on farmed red deer indicate the majority (> 90%) of calf losses can be collectively attributed to the following causes: starvation/dehydration (mismothering); dystocia (birth injury); misadventure; nonviability (low birth weight); and infectious agents (Asher & Adam 1985, Gill 1985). 'Mismothering' is a euphemism for calf abandonment leading to early starvation/dehydration of the calf. It is probably exacerbated in pastoral farming systems in which hinds must compete for ideal and isolated birthing sites. Such competition can lead to severe disruption of the dam–calf bonding process immediately after birth. It has also been argued that cases of dystocia, involving a protracted and difficult parturition, and misadventure (e.g., calves migrate far from their birth sites) may also be increased due to hind–hind disturbance effects in high stocking intensity environments (Asher & Pearse 2002). Therefore it becomes apparent that a significant source of wastage through perinatal mortality is attributable to the artificial environment associated with the high intensity farming of a species that has yet to be fully domesticated. Current management practices for farming red deer in New Zealand strongly focus on providing improved calving environments to meet the behavioural needs of the species, particularly with respect to lowering stocking intensities around the birthing period and the provision of low vegetative cover for the concealment of calves within their first few weeks of life.

As deer farming intensifies, the list of potential pathogens that potentially influence calf survival grows. Although the overall recorded incidence of calf mortality through infectious agents appears relatively low (albeit that the original studies were conducted over 30 years ago), the sporadic nature of specific infections can have disastrous consequences on reproductive productivity of individual farms. High perinatal death rates (i.e., >10%) among red deer calves on New Zealand farms have occurred from acute outbreaks of *Cryptosporidia* and *Salmonella* (Asher & Pearse 2002).

Conclusions

Clearly, there is no one 'model' species that defines typical cervid reproductive cycles, attesting to the complex array of life histories and reproductive strategies of the cervids in general. Our current understanding of reproductive biology largely centres on large bodied, temperate (and highly seasonal) species that have a close association with man. In contrast, reproductive cycles of the smaller bodied, tropical (aseasonal) species are generally poorly understood, with this group represented by many taxa that are at serious risk of extinction. However, the development of large scale deer farming, in particular of red deer in New Zealand, has provided research opportunities that are starting to unravel some of the complex life histories and adaptations of key cervid species.

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Laboratory and Pet Rodents, and Lagomorphs

MOLLY VARGA AND TIM MORRIS

The vast majority of small mammals (Fig. 40.1) presented to veterinarians will be kept as pets rather than animals being bred for the pet trade or other activities, such as biomedical research. Such larger breeding activities will require more of a colony management approach; this is described in Chapter 41 and for research applications by Suckow et al (2012).

With the exception of rabbits and ferrets, most of these small pet mammals are not routinely neutered, so reproductive disease is a common presenting problem. With some species, particularly those that have been used in biomedical research, much is known about the reproductive cycle, its manipulation, diseases, and disorders. However, when the species in question has only recently been introduced into the pet trade (for example, the sugar glider or African pygmy hedgehog), the knowledge base is sparse and is often reliant on largely anecdotal evidence. The species of small pet mammals covered in this chapter will include many kept around the world but also reflects the experience of the authors.

Sexing

The ability to distinguish between male and female animals is equally important when presented with individuals or in breeding facilities.

Rodents

Sexing myomorph rodents: (rats/mice/hamsters/gerbils) see Figs 41.1–41.6. In males the distance between the genital papilla and the anus is greater than in females. This is easy to determine in adults but more challenging in younger animals. Males of all species possess a true scrotum, and in mature animals the testes are easily identified. Females have separate urinary and genital tract openings; the genital opening is positioned between the anus and the urinary tract opening. Being able to compare individuals of known gender can be very helpful when attempting to determine an animal's sex (often known colloquially as 'sexing').

Sexing hystricomorph rodents: (chinchillas/guinea pigs/degus) see Fig. 41.1. These are more challenging to sex. Although the anogenital distance in the male is always greater than in the female, differentiation of the genitalia is more difficult. Male guinea pigs do not have a scrotal sac, and the testes are held against the body wall in a prescrotal sac. The genital opening in both sexes appears similar, with the shape of the anogenital opening in male guinea pigs being described as an 'I' and in the female as an inverted 'Y'. Female chinchillas have a vulvar papilla that can resemble a penis. The anogenital distance is slightly greater in the male than in the female, and the opening of the genital papilla is slit-like in females and circular in males. Male chinchillas do not have a defined scrotal sac, and the inguinal ring remains open, thus the testes can be retracted and are not always obvious. Degus are similar in appearance to chinchillas; males do not have a scrotal sac, and the anogenital distance is greater than in females. Guinea pigs, chinchillas, and degus have a vaginal closure membrane, which is only open for a few days during oestrus and at parturition.

Rabbits

Rabbits can be challenging to sex, particularly when young. The distance between the genital papilla and the anus is not greater in the male than the female (Bishop 2002). In most males, gentle pressure will extrude the end of the penis; the genital opening is circular and not slit-like as it is in the female. Unlike many other small mammals, the rabbit testes are cranial to the penis. In older neutered male rabbits, particularly those that are overweight, it can be difficult to extrude the end of the penis, making gender determination slightly more challenging.

Ferrets

In male ferrets (often known colloquially as 'hobs') the distance between the genital opening and the anus is greater than in females; the prepuce is located on the ventral caudal abdomen, with the testes located ventral to the anus. The vulva is ventral to the anus in females and encloses both the genital and urinary openings. When the vulva is engorged in a female in oestrus, it can be mistaken for testes, although gender is easy to determine in this species.

African Pygmy Hedgehogs

In African pygmy male hedgehogs, there is a greater distance between the genital opening and the anus compared with females; in fact, the prepuce is located on the ventral midabdominal surface. There is no defined scrotal sac, so the testes may not be immediately obvious. It can be difficult to visualise the genitalia, because these animals will roll up into a 'tight ball' when frightened. To overcome this, they can be placed in a clear glass or Perspex box through which

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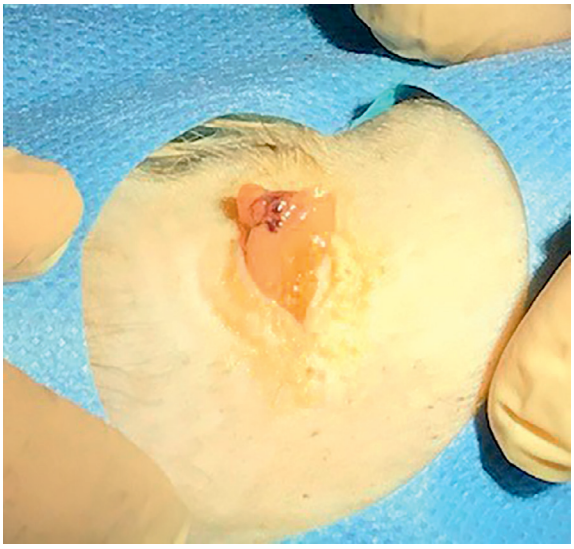
Order Rodentia
- Suborder Hystricomorpha
  - Infraorder Hystricognathi
    - Parvorder Caviomorpha
      - Family Cavidae – includes guinea pig
      - Family Chinchillidae – includes chinchilla
- Suborder Myomorpha
  - Superfamily Muroidea
    - Family Cricetidae – includes hamster
    - Family Muridae – includes rat, mouse, gerbil

Order Lagomorpha
- Family Leporidae – includes rabbit

• **Fig. 40.1** Classification of common rodents and rabbits.



• **Fig. 40.2** Surgical approach to expose the ovaries of a rat.

they can be viewed, thus avoiding the need for general anaesthesia, which otherwise would be necessary for this simple procedure.

Sugar Gliders

Male sugar gliders have obvious testes on the midventral abdomen, rostral to the urogenital opening/cloaca. Female sugar gliders have a ventral pouch, which opens in the midventral abdomen.

Anatomy

Rodents

Myomorph Rodents

In females the urinary and the genital tract openings are completely separate. The ovaries are located caudal to the kidneys and are readily accessed for surgical ovariectomy (Fig. 40.2). The uterus has two horns (bicornuate), with a short uterine body, single cervix, and vagina; the rat is different, with two uterine horns and a double cervix, each of which opens into a single vagina. Male myomorph rodents have large testes compared with body size; however, because the inguinal ring remains open, they can still be retracted towards the abdomen from the clearly defined scrotum. Accessory sex glands in the male comprise the prostate, seminal vesicle and coagulating glands, bulbourethral glands, and ampullary glands; there is an os penis present in male rats. Both male and female rats and mice have significant amounts of mammary tissue, which is very different from hamsters and gerbils, in which mammary tissue is more limited.

Hystricomorph Rodents

All female hystricomorph rodents have a vaginal closure membrane, which seals the end of the vagina except during oestrus and at parturition. The paired ovaries are located caudal to the kidneys. The uterus is bicornuate, terminating in a single uterine body and a single cervix in the guinea pig, whereas in the chinchilla the uterus is duplex, with two cervices present. Male hystricomorph rodents always have an open inguinal canal from which the testes can be retracted; there is no true scrotum, and the testes are held close to the body wall. Male guinea pigs have an os penis. Accessory sex glands include the seminal vesicles, bulbourethral gland, copulatory glands, and prostate gland. In male guinea pigs, the seminal vesicles are long and coiled and situated intraabdominally; they can be mistaken for uterine horns.

Rabbits

Female rabbits have paired ovaries, caudal to the kidneys; the uterine horns are long and curved, terminating in two cervices (there is no uterine body) and a single vagina. The urethral opening is found on the ventral wall of the vaginal vault. In male rabbits the inguinal canal remains open, and in mature males the testes are located in a true scrotal sac cranial to the prepuce. The accessory sex glands comprise the proprostate, the prostate, the paraprostates, and the bulbourethral glands.

Ferrets

In female ferrets, paired ovaries are located in the dorsal abdomen, caudal to the kidneys; the uterus is bicornuate, ending in a short uterine body and a single cervix. The urethral orifice is found on the floor of the vaginal vestibule so that the genital and urinary tract openings are not separate. In male ferrets the testes are located ventral to the anus in a defined scrotal sac. The inguinal ring remains open, and the testes are partially retracted somewhat during winter, when it is not breeding season. The prepuce is located on the ventral midabdomen, and there is a hook-shaped os penis that supports the glans penis. The prostate is the only accessory sex gland in male ferrets.

African Pygmy Hedgehogs

The female has paired ovaries caudal to the kidneys in the dorsal abdomen. The uterus is bicornuate with no defined uterine body, the horns terminating in a single cervix. The male has a 'snail-shaped' penis, with lateral projections on each side of the external meatus; there is no os penis. The testes are found within the lower abdomen in paraanal recesses (Bedford et al. 2000).

Sugar Gliders

Female marsupials have a reproductive tract much smaller, relative to body size, compared with placental mammals, because the

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young are born much earlier in the developmental process. Paired ovaries are located in the dorsal abdomen caudal to the kidneys. There is a paired uterus, each ending in a vaginal cul-de-sac, which is divided into right and left sides by a median septum. Each vaginal cul-de-sac is joined caudally to a median vagina through which the joey is expelled, as well as two lateral vaginas; all three vaginas terminate in a urogenital sinus that also contains the urethral opening. The male has a forked (bifid) penis; the urethral opening is at the proximal end of the penis, not at the distal tip of the fork; the forked penis is complimentary to the lateral vaginas during copulation. The prostate and the bulbourethral glands are poorly defined; there are no seminal vesicles or coagulating glands. The testes are present in a clearly defined scrotum situated on the ventral midabdomen.

Normal Reproductive Cycles

This information is described in Chapter 41, including key reproductive biological data for rodents and other species in Table 41.1.

Reproductive Diagnostics for Small Mammals

Vaginal Cytology

Examination of exfoliate vaginal cytology is a useful diagnostic procedure in a range of species. It confirms the integrity of the hypothalamus–pituitary–ovarian axis within the individual (Goldman et al. 2007) and enables identification of the stage of the oestrous cycle in a particular individual. The appearance of the stained vaginal epithelial cells correlates with changes in the circulating concentrations of reproductive hormones. In hamsters, due to the presence of vaginal pouches that retain sloughed squamous cells and leucocytes, vaginal cytology as determined by smears can be misleading, as even animals that are not in oestrus may still have cells typical of oestrus retained within the pouches. Vaginal cytology is of limited use in hystricomorph rodents because, for much of the cycle other than at oestrus, they have a vaginal membrane that 'closes off' the vagina. Vaginal cytology has not been validated in sugar gliders.

Ultrasonography

B-mode transabdominal ultrasonography can be valuable both in the diagnosis of pregnancy and in the evaluation of the reproductive tract, in both the male and the female. For smaller animals, a 10 MHz probe is preferred. Alternatively, a 'stand-off' can be used with a larger probe, although this can make examination challenging.

Endocrine Markers

Many commercial laboratories are now able to offer assays of reproductive hormones for many species. This is useful in ferrets and rabbits to show the presence of ovarian remnants or ectopic ovarian tissue (ovarian remnant test) in females and to confirm cryptorchidism ('rig test') in males, as well as for wider uses such as for other steroid hormones in adrenal disease that is associated with neutering (Wagner et al. 2005). The use of blood hormone assays in order to predict the optimum time for breeding is not commonly performed in these species; assays of reproductive hormones are not routinely used to confirm pregnancy.

Noninfectious Diseases

Diseases due to poor husbandry and feeding are relatively common in small mammals kept as pets, often affecting reproductive function.

Husbandry and Environment

Husbandry

Ensuring the correct husbandry for a particular species is important to ensure reproductive health; for example, hyperthermia has been shown to cause reduced sperm production, as well as affecting fetal development. In addition, it can also depress lactation in various rodent species (Hansen 2009). Studies have shown that overcrowding increases the time to first pregnancy and reduces the number and weight of the first offspring in mice (Burroni et al. 2014). Odours from other individual animals, particularly associated with overcrowding, can affect fertility. The odour of male mice induces oestrus in female mice, whereas the odour from a novel male can result in pregnancy failure in recently impregnated female mice. Shah et al. (1980) demonstrated that overcrowding affected the production and/or release of GnRH from the hypothalamus of male rats, resulting in impaired reproductive performance.

Social and Physical Environment

Meeting species-specific behavioural, social, and physical environmental requirements optimises reproductive capability, as animals that have their welfare needs met will demonstrate normal behaviour while being free from discomfort, distress, and disease (Whitaker et al. 2009). The scope of what is 'normal behaviour' varies between species, as do their environmental requirements, social as well as in their husbandry (Wolfe 2005). Exposure to certain electromagnetic fields has been linked to infertility in male rodents; for example, Kim et al. (2007) showed that electromagnetic fields affected proliferation and differentiation of spermatogonia in male rats.

Nutritional Problems Affecting Reproduction

Major Nutrients

Dietary protein requirements are difficult to quantify in those species that are 'hindgut fermenters', such as rabbits, guinea pigs, chinchillas, and degus, due to the bacterial synthesis of amino acids in the caecum and the reingestion by either caecotrophy or coprophagy of protein-containing faeces. In species such as the ferret, which has relatively short small and large intestines, dietary protein levels are key to good health, and levels that are too low will affect their fertility. However, there are few specific reports linking dietary protein and fertility in many of these species. Diets low in fat delay puberty in female rats (Frisch et al. 1977), leading to the conclusion that a minimum level of body fat is required for normal reproductive cyclicity in rodents.

Vitamins

Hypovitaminosis A has been shown to suppress spermatogenesis in male rats (Huang & Hembree 1979). Hogarth and Griswold (2010) demonstrated that when male rodents are vitamin A deficient, spermatogenesis ceases. Maternal vitamin A deficiency can lead to congenital malformations of offspring in many rodent species (Wilson & Warkany 1948). For example, rats with induced hypervitaminosis A displayed decreased testicle mass, focal lesions in the

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seminal tubule epithelium, and reduction in the relative volume of interstitial tissue and in the nuclear volumes of Leydig cells (Lamano et al. 1978). In female rats, placental agenesis, retarded embryonic growth, and eventual embryonic death occurred as a result of hypervitaminosis A (Love & Vickers 1976).

Vitamin C has a role in hormone synthesis, as well as protection from oxidative damage (Luck et al. 1995). Vitamin C levels significantly affect the function of both ovaries and testes. For example, its supplementation increased spermatogenesis and plasma testosterone levels in Wistar rats (Sönmez et al. 2005), as well as protecting spermatogenesis, sperm integrity, and fertility; thus hypovitaminosis C has multiple detrimental effects on fertility. It also prevents oxidative stress injury to sperm, and its antioxidant effects are closely linked with those of vitamin E.

Halloran and DeLuca (1980) examined the effects of hypovitaminosis D on the reproductive capabilities of female rats. They showed reduced fertility (by 75%), diminished litter size (by 30%), and impaired neonatal growth, although reproduction was not halted. It is not known whether it is the lack of vitamin D or the hypocalcaemia associated with hypovitaminosis D that is responsible for the observed effects. Male rats that had hypovitaminosis D showed fewer successful matings (43% less) and fewer successful pregnancies (73% less) compared with rats with sufficient vitamin D (Kwiecinski et al. 1989).

Vitamin E (along with vitamin C and selenium) is intricately involved in normal fertility. Hypovitaminosis E in pregnant female rats leads to fetal death and resorption and lack of spermatogenesis in male rats (Burton 1994). Vitamin E supplementation has been shown to protect rat testes against the oxidative damage caused by ozone (Jedlinska-Krakowska et al. 2006) and to improve sperm motility and viability (Agarwal & Said 2005).

Minerals

In male rats, selenium deficiency results in reduced circulating testosterone concentrations and testicular atrophy (Behne et al. 1996); in addition, active spermatogenesis was reduced or absent (Wu et al. 1973). Selenium deficiency in female rats results in infertility, abortions, and placental retention (Bedwal & Bahuguna 1994).

Zinc deficiency leads to impairment of angiotensin-converting enzyme and subsequent depletion of testosterone and reduced spermatogenesis; there is an increase in abnormal sperm (Millar et al. 1958). In the female, zinc deficiency results in reduced FSH/LH production, abnormal ovarian development, and disruption to the oestrous cycle. Abortion, prolonged gestation, stillbirth, dystocia, and low birth weight of offspring are also linked to zinc deficiency in rodents.

Disorders of the Oestrous Cycle

Cystic Endometrial Hyperplasia

Cystic endometrial hyperplasia (CEH) is defined as progesterone-induced hyperplasia of the endometrium, with cystic dilatation of the endometrial glands. Progesterone from the corpora lutea causes an increase in endometrial gland secretory activity, as well as a reduction in myometrial contractility. Thus when there is a closed cervix, secretions can accumulate within the uterine lumen, leading to hydrometra (in which the secretion is largely serous), and mucometra (in which the secretion is more mucoid). In both hydro- and mucometra, the internal environment of the uterus is sterile. Increased oestrogen secretion during folliculogenesis causes the cervix to dilate, providing a route for bacterial invasion of the uterus. If these bacteria become established, then pyometra develops. Oestrogen increases the number of progesterone receptors in the endometrium, so that with each subsequent cycle, there is likely to be an enhanced response to progesterone. CEH and hydro- and mucometra do not always result in the development of pyometra because it can develop independently of these conditions. Initially, affected animals may be asymptomatic, and although subsequently they may still only show nonspecific signs, eventually, affected females will develop increased abdominal girth and possibly become anorexic. Diagnosis is initially by abdominal palpation with subsequent ultrasound confirmation; treatment is by ovariohysterectomy. Fluid-filled uterine horns present an increased risk of uterine torsion, and the associated acute abdominal pain may be the primary clinical sign and the primary reason for an owner seeking a veterinary consultation.

Hydrometra

Hydrometra in ferrets is strongly associated with the presence of ovarian neoplasia (Jekl & Hauptman 2017).

Ovarian Cysts

Ovarian cysts occur in many species and have been reported in rabbits, guinea pigs, chinchillas, gerbils, rats (Fig. 40.3), golden hamsters (Fig. 40.4), and mice; it is likely that they also occur in other species. Ovarian cysts occur very commonly in the guinea pig and may cause anorexia, bilateral symmetrical alopecia, and a reduction in fertility, although some sows are asymptomatic. Three types of cysts have been identified in guinea pig ovaries: serous cysts (cystic reti ovari), which are the most common form; rarely, follicular cysts; and parovarian cysts. Serous cysts are present throughout the oestrous cycle, whereas follicular cysts were less common during dioestrus. Abnormal increases in FSH secretion have been suggested as a cause of cystic ovarian disease in the guinea pig. Diagnosis is often possible by palpation alone; however confirmation using ultrasonography is useful (Beregi et al. 2001). Treatment with human chorionic gonadotrophin (hCG) is successful in some cases in which the cyst is sensitive to the LH-like action of hCG; however, in those cases that are refractory to medical treatment, ovariectomy/ovariohysterectomy is the treatment of choice.

• **Fig. 40.3** Ovarian cyst in rat surgically exposed for ovariohysterectomy.

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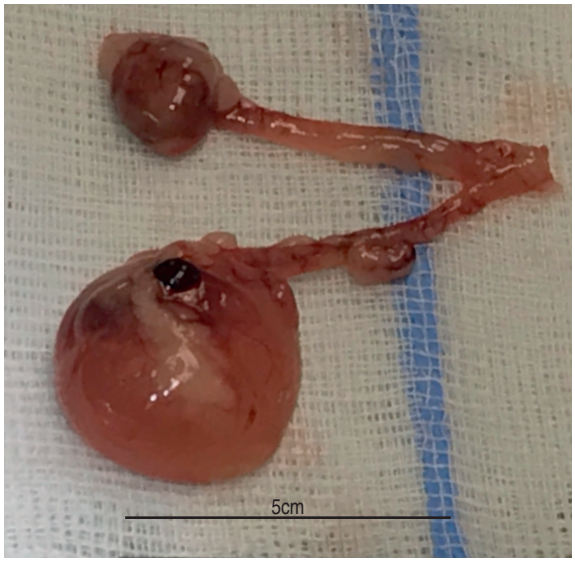

• **Fig. 40.4** Hamster ovarian cyst and uterus after excision.

Hyperoestrogenism in Ferrets

Ferrets, in common with many other small mammals, are induced ovulators. Without cervical stimulation from copulation, the follicles do not ovulate and, subsequently, frequently become luteinised. However, the anovular follicles frequently persist, remain, and continue to produce oestrogens. Approximately 50% of intact female ferrets, particularly those kept alone or with other females, show signs of prolonged oestrus (Jekl & Hauptman 2017). High circulating oestrogen levels cause bone marrow suppression and reduced extrasplenic haematopoiesis, often resulting in profound anaemia, thrombocytopenia, and leucopenia; the disease can prove fatal if not treated. Clinical signs include lethargy, weight loss, prolonged signs of oestrus with vulvar enlargement, pale mucous membranes, and petechiae. Diagnosis is based on a history of prolonged oestrus, with clinical signs of an enlarged vulva and pale mucous membranes; laboratory samples provide confirmation of nonregenerative anaemia and thrombocytopenia/leucopenia. Treatment includes both the termination of oestrus and correction of anaemia; the latter can be terminated medically in various ways. Administration of proligestone (colloquially known as 'jill jab') has been used successfully for many years, whereas hCG (100 IU per ferret; two doses, 7 days apart) can be used to induce ovulation with subsequent luteinisation. GnRH can also be used to stimulate an endogenous LH surge. In early oestrus (first 1–2 weeks), Deslorelin implants can be used, although the use of implants later in oestrus may exacerbate the bone marrow suppression that is already occurring.

Fetal Resorption, Abortion, and Stillbirth

Periods of poor nutrition, stress, and ill health can all lead to failure of a pregnancy; the timing of the insult defines the outcome (resorption or abortion). Various infectious agents, such as Chlamydia, *E. coli*, *Pasturella* spp, *Salmonella* spp, and Toxoplasma, may be implicated (see later section 'Infectious Disorders'). A thorough nutritional and husbandry review is essential, particularly when several females have lost litters within a short time period. Samples should also be submitted for bacterial culture and supportive care offered to the females, pending the laboratory results. Zoonotic aspects should also be considered.

Periparturient Problems and Dystocia

Uterine Rupture in Rabbits

Uterine wall rupture occurs regularly in breeding rabbits and may be a coincidental finding. It is difficult to diagnose other than at surgery, and it may be implicated in some cases of dystocia. Treatment is surgical, and although repair of the uterine wall is possible, ovariohysterectomy is the preferred method. Ectopic abdominal pregnancies in may also occur in rabbits without uterine rupture (Gil et al. 2004).

True Dystocia

This is uncommon in most small mammals because, in most of these species, large numbers of small size offspring are normally born. In those species that have fewer, precocial offspring (e.g., guinea pig or chinchilla), then there is a higher risk of dystocia. Prolonged labour, particularly with unproductive straining, should be a cause for investigation. Radiography or ultrasonography will confirm pregnancy and show the disposition of the retained young, allowing assessment of whether vaginal birth is possible or a Caesarean surgery is preferable. Obesity, large litters resulting in maternal exhaustion, fetomaternal disproportion, faulty disposition, and ectopic pregnancies are all possible causes. The guinea pig presents an additional challenge with respect to dystocia, in that, theoretically, if a sow is not bred before 6 months of age, her symphysis pubis bones will have fused, making normal parturition much less likely. The author (MV) has found this does not always occur as several older nulliparous female guinea pigs have had a normal parturition. Similarly, it has been suggested that the pubic symphysis fuses in female African pygmy hedgehogs at around 1 year of age, leading to the suggestion that this species may also suffer from dystocia if breeding for the first time occurs after this time. This is anecdotal, and there are no confirmed reports of dystocia from this cause in the literature.

Eclampsia and Pregnancy Toxaemia

Preeclampsia

Preeclampsia develops in the later stages of pregnancy. It has been reported clinically in rabbits, ferrets, and guinea pigs; mice and guinea pigs are used as experimental models of preeclampsia in humans. Fetal factors and the pressure of the weight and size of the gravid uterus occlude the blood vessels of the uterus, leading to ischaemic necrosis, hypertension, proteinuria, and elevated creatinine levels. Frequently, there are no specific clinical signs, only nonspecific ones, with the pregnant female becoming increasingly unwell towards the end of gestation; it can be difficult to distinguish it from pregnancy-related ketosis (pregnancy toxaemia). Its presence can be confirmed by elevated blood creatinine levels and proteinuria. Treatment is emergency Caesarean surgery; however, the prognosis is poor.

Eclampsia

Eclampsia is defined as preeclampsia complicated by maternal seizures. Hamsters in late gestation display a disease very similar to eclampsia in humans. Affected hamsters have fibrin thrombi in the capillaries, disseminated intravascular coagulation, and demonstrate severe changes to the renal glomerulae and tubules. The collapse of normal renal function leads to azotaemia and seizures (Barthold et al. 2016, Richter et al. 1984). Cases of true eclampsia

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are rare; however, a history of seizure, in combination with acidosis and ketosis, is pathognomonic. Treatment is the same as for preeclampsia, with additional control of the seizures and support of renal function. Prognosis is generally very poor and euthanasia should be considered.

Pregnancy Toxaemia

Pregnancy toxaemia is a metabolic condition that usually affects obese females in late pregnancy or early lactation. A combination of factors is thought to be involved, namely: inability to meet nutritional needs due to the size of the gravid uterus; the energy drain placed on the dam by lactation; environmental factors; and other stressors. This results in a negative energy balance, and the animal becomes ketotic and acidotic. Often affected females are very poor bodily condition and are likely to be dehydrated, possibly recumbent, and are at risk of hypoglycaemic seizures. Diagnosis is by confirmatory blood analysis, demonstrating the presence of acidosis, ketosis, and hypoglycaemia; urine analysis shows a low pH (5–6 rather than the normal 9), ketonuria, and proteinuria. Cases of preeclampsia can present in a similar way. However, pregnancy toxaemia can be differentiated from preeclampsia by the presence of ketones in acidic urine; treatment is difficult and is based on the provision of fluids and correction of hypoglycaemia with frequent small feeds of a suitable support diet (Proença & Mayer 2014). It is important not to feed too much at once, as it causes a blood sugar 'spike', consequential insulin release, and the potential for hypokalemia and hypophosphataemia as glucose is forced intracellularly.

Retained Fetal Membranes

Fetal membrane retention can lead to acute metritis and septicaemia. Clinical signs include malaise, anorexia, and serosanguinous to purulent vaginal discharge; diagnosis is confirmed by a history of recent parturition, with consistent clinical signs. Treatment can be attempted medically; the use of aglepristone to cause uterine contractions and expulsion of the fetal membranes has been reported in lagomorphs and rodents (Gogny & Fieni 2015), or alternatively, oxytocin could be used to achieve the same aim. Supportive care and antibiosis are also indicated; however, care needs to be taken not to adversely affect any young still being suckled and to avoid the risk of antibiotic-induced enterocolitis (Morris 1995). Alternatively, ovariohysterectomy can be considered.

Vaginal Prolapse

This is rare but has been reported in rabbits and chinchillas. Prolapse usually occurs within a few days of parturition, and one report of a cluster of cases in related females suggests a genetic predisposition in rabbits (Becha et al. 2006); it has also been reported in postpartum guinea pigs and chinchillas (Kondert & Mayer 2017). In some instances, the uterus also prolapses. Typically, a postpartum female will be observed to be straining vigorously with a soft tissue mass visible at the vulva. Treatment of a simple nontraumatised prolapse includes cleaning and replacing the prolapse tissues and placing retention sutures in the perivulvar tissue to prevent reoccurrence. When there is a more severe prolapse or when there is significant soft tissue damage, ovariohysterectomy is the treatment of choice. The more extensive the prolapsed and the more tissue damage sustained, the worse the prognosis; in extreme cases, bladder prolapse may be associated with uterine prolapse in the rabbit (Harcourt-Brown 2017). These cases are challenging: the bladder must be gently replaced, and any necrotic or damaged portions resected, in addition to removing the genital tract. The prognosis in these cases is poor.

Neoplasia

Table 40.1 summarises the reported neoplasms of the reproductive tract in different species (Laik-Schandelmaier et al. 2017, Jekl et al. 2011, Goodman et al. 1979, Suárez-Bonnet et al. 2010). Many neoplasms of reproductive tissue origin are initially hormone-induced, although eventually the tumour will become self-sustaining, and removal of hormone stimulation (medically or surgically) will not arrest its growth. Equally, some neoplasms will themselves produce hormones, which then will affect various other parts of the genital tract. Clinical signs related to neoplasia of the genital tract include the presence of space-occupying lesions, abnormal reproductive activity, and bleeding from the genital tract.

Miscellaneous Reproductive Disorders

Adrenal Disease in Ferrets and Rabbits After Neutering

Adrenal disease commonly occurs in ferrets, and it is also increasingly being diagnosed in rabbits (Wagner et al. 2005, Lennox & Chitty 2006). Initially recognised in the United States, where ferrets are routinely desexed at 6 weeks of age, adrenal disease is caused by the failure of negative feedback on the hypothalamic–pituitary–gonadal axis after neutering. In most species, removal of the gonads initially leads to FSH and LH remaining elevated in the circulation because with the absence of organs that have receptors for these hormones, there is no negative feedback. High FSH and LH levels then depress the production of GnRH by a negative feedback loop. The reduction of GnRH leads to lower levels of LH and FSH in the blood, and the lack of gonadal tissue does not provide a continued stimulus for GnRH production. In ferrets, GnRH continues to be produced, possibly related to prolonged photoperiod; this negative feedback loop fails, resulting in chronically high LH and FSH levels in the blood. Chronically high LH and FSH levels stimulate the expression of LH receptors on the adrenal gland. These receptors, when stimulated by LH, lead to the production of active hormones, which in turn result in clinical signs being displayed (vulvar or prostatic enlargement, mammary development, bilateral symmetrical alopecia, atypical aggression in rabbits). Initially, the adrenal gland is hyperplastic; however, this progresses to an adenomatous change and eventually to adenocarcinoma.

Diagnosis is based on clinical signs (usually but not exclusively) in a neutered animal and the confirmation of elevated hormone levels (adrenal panel: oestrogen, 17-OH progesterone, and androstenedione; Wagner et al. 2005). Treatment can be either medical or surgical; the former involves implantation with Deslorelin, a GnRH agonist, which causes initial up-regulation of and then negative feedback on the hypothalamic–pituitary axis. Depending on the dose and season of the year of implantation, the effects can last 18 to 48 months. It is important to note that, in cases that have already progressed to malignancy and the tumour does not require hormonal stimulation to continue growing, implantation will only reverse the visible clinical signs. As a consequence, the malignancy will progress and must be treated separately. Surgical treatment, which involves the removal of one or both adrenal glands, is difficult, particularly because the left adrenal gland is

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TABLE 40.1 Summary of Reported Neoplasms of the Reproductive Tract in Small Pet Mammals

Species	Ovary	Uterus	Cervix	Vagina	Mammary Gland	Testicles	Prostate/Accessory Sex Glands	Penis
Guinea pig	Granulosa cell tumours, teratomas, embryonic placentoma	Fibromas, leiomyoma, myxosarcoma, leiomyosarcoma	Adenoma and adenocarcinoma		Mammary adenocarcinoma, mammary gland adenoma, mixed mammary tumour	Embryonal carcinoma		
Chinchilla								
Degu		Angioleiomyoma		Vaginal leiomyosarcoma				
Rat	Tubular adenoma, tubular adenocarcinoma, malignant granulosa cell tumour, ovarian mesothelioma	Leiomyoma, uterine endometrial stromal polyps			Mammary fibroadenoma (female Sprague Dawley rats). Prolactin responsive. Adenocarcinoma	Interstitial cell tumours, mesothelioma, Sertoli cell tumour, Leydig cell tumour, preneoplastic focal hyperplasia		
Mouse	Papillary cystadenomas Ovarian tubular adenomas, granulosa cell and thecal cell tumours	Haemangioma and haemangiosarcoma Uterine endometrial stromal tumours, uterine stromal polyps, uterine adenocarcinoma, uterine wall leiomyosarcoma Histocytic sarcoma			Mammary gland adenocarcinoma (retrovirus: mouse mammary tumour virus)	Teratomas	Sebaceosquamous adenomas and carcinomas	
Hamster	Ovarian thecal cell tumours, granulosa cell tumours	Uterine adenocarcinoma			Mammary adenocarcinoma	Rarely reported		
Gerbil	Granulosa cell tumour, dysgerminomas, luteal cell tumours	Leiomyoma, uterine adenocarcinoma, oviduct adenocarcinoma			Mammary neoplasia rarely reported			
Rabbit		Uterine adenocarcinoma			Mammary dysplasia associated with prolactin producing pituitary adeonomas, mammary adenocarcinoma, leiomyoma, leiomyosarcoma	Seminoma, teratoma	Prostatic adenoma	
Ferret	Gonadostromal tumour, ovarian teratoma, thecoma, ovarian cystadenocarcinoma	Uterine teratoma, uterine leiomyoma			Papillary cystadenocarcinoma	Interstitial cell tumour, Sertoli cell tumour	Preputial gland tumour	
African Pygmy Hedgehog	Granulosa cell tumour	Uterine spindle cell tumour, leiomyoma, endometrial polyps, uterine adenosarcoma, endometrial stromal cell sarcoma, uterine adenocarcinoma			Mammary gland adenocarcinoma, papillary adenoma, mammary gland carcinoma			

closely associated with the vena cava. Removal of the adrenal gland will necessitate subsequent supplementation of corticosteroids.

Paraphimosis in Chinchillas

Paraphimosis – fur ring – in chinchillas is a condition commonly seen in male chinchillas. Clinically, the penis may be protruding or the animal is observed to be excessively grooming the perineal area. In these cases, a ring of fur becomes trapped around the base of the penis, limiting its retraction into the prepuce. The penis tip may become engorged and necrotic, and there is a risk of urethral obstruction. Treatment involves identification and removal of the fur ring and application of hypertonic glucose solution to the penis tip if it is very swollen. In cases in which the tip of the penis is necrotic, amputation should be considered.

Pseudopregnancy

Pseudopregnancy occurs in many small mammalian species, in both nonmated or in those which are mated but do not conceive. As a consequence, the subsequent luteal phase, although not supporting a pregnancy, does stimulate mammary development, as well as hyperplasia of the endometrium. As the luteal phase ends, the progesterone concentrations decline and prolactin concentrations increase, resulting in behavioural changes and sometimes lactation. Excessively long luteal phases may be seen in some pseudopregnant laboratory rodents. Treatment is not always necessary because pseudopregnancy is a normal occurrence, and once the corpora lutea regress, either normal cyclicity returns or the animal becomes anoestrus or progresses to oestrus. If the animal is lactating, then treatment is warranted, and cabergoline can be used to good effect. If repeated pseudopregnancy occurs, then the animal should either be mated or spayed.

Uterine Venous Aneurysms

Originally reported in three New Zealand white rabbits in 1992, uterine venous aneurysms are one potential cause of haematuria in the female rabbit. Varices and aneurysms of uterine subserosal and myometrial venous plexi are reported in other species, whereas endometrial aneurysms are not. These fragile blood vessels rupture and bleed into the lumen of the uterus. Clinical signs include vulva bleeding and haematuria, which may be associated with significant blood loss; however, the signs are episodic, not consistent. Treatment is ovariohysterectomy.

Uterine Torsion

Uterine torsion has been reported in a variety of species and is associated with a distended uterus (due either to gravidity or hydrometra). Affected animals may be presented in acute pain or, more commonly, collapsed and in shock; treatment is ovariohysterectomy. Prognosis is dependent on the condition of the animal before surgery, but it is generally guarded to poor.

Cryptorchidism

Cryptorchidism is the failure of the testes to descend through the inguinal canal and into the scrotal sac. The testes may remain intraabdominal, may have partially descended into the inguinal ring, or may have progressed through the inguinal ring but not into the scrotum. Retained testes (particularly in species with a defined scrotum and lower testicular temperature) may become neoplastic. Castration is indicated.

Testicular Evisceration

This is a relatively common occurrence subsequent to fighting in male rabbits. Animals will be presented with bleeding from the scrotum, or scrotal contents will be either be observed protruding from the scrotum or completely removed from the scrotum after an altercation. Treatment depends on the severity of the injuries, but it may be castration or removal of the remaining tissue with analgesia and antibiosis. Occasionally, rabbits are presented with a single testis, the missing one having been removed previously during a fight; in such animals, it can be difficult to determine whether the animal is cryptorchid or not. There may be an obvious scar where the scrotum was torn, but if not, then a hCG stimulation or 'rig' test (see previous mention) can be performed to avoid the need for exploratory laparotomy with its attendant risks of adhesion formation postoperatively.

Infectious Disorders

Bacterial

Treponema paraluis cuniculi

Treponema paraluis cuniculi is a spirochaete organism, which causes venereal syphilis in rabbits (DiGiacomo et al. 1984). It can be transmitted either by mating with an affected individual or via direct contact of kits with an affected doe; asymptomatic carriers/shedders exist within the population. *Treponema* infection manifests as papules, vesicles, ulcers, and crusting around the external genital organs and, occasionally, the eyes/nose and perineum; diagnosis can be made on clinical signs and confirmed by serology (Saito et al. 2003). Diagnosis can be aided by an ELISA test, which is available commercially, dark field microscopical examination of scabs and exudate demonstrating helical organisms, or histopathology. Treponematosis can be associated with an unwillingness to mate and abortions in affected does. Treatment is three times a week doses of procaine/benzathine penicillin. Response to treatment is rapid and is often noted after the first dose of medication; differential diagnoses include atypical myxomatosis and autoimmune skin disease.

Pasteurellaceae

Pasturella multocida and *Pasturella pneumotropica* are common pathogens of rabbits and other small mammals. Depending on the route of transmission (aerosol, direct contact, haematogenous, sexual transmission), these bacteria can cause varying clinical signs. *P. multocida* has been associated with balanoposthitis, metritis, pyometra, and mastitis in rabbits, whereas *P. pneumotropica* has been associated with mastitis, chronic metritis, infertility, bulbourethral gland infection, and abortion in rats. These bacteria less frequently affect other species. Diagnosis is by bacterial culture, and treatment depends on the clinical signs noted. Abscessation is generally better treated surgically in rabbits and rodents in particular, and infections involving the genital tract can be managed by neutering once it is certain that there is no infection elsewhere.

Staphylococcus

Staphylococcus aureus infections occur commonly in a variety of species. *S. aureus* is often opportunistic and may be carried subclinically in the nasopharynx. Transmission is usually by direct contact

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or environmental contamination, although aerosol transmission is possible. Clinical signs can include mastitis, abscessation in the preputial glands, and suppurative lesions within the genital tract of both sexes. 'Young doe syndrome' is also associated with *S. aureus,* which manifests as a toxaemia, in which young nursing does develop a fever, with anorexia and swollen lymph nodes; potentially, this infection can be fatal. Diagnosis is confirmed by bacterial culture, and treatment is based on these results. Abscesses are often best treated surgically.

Mycoplasma

Mycoplasma pulmonis infection is common in pet rats and mice and can occasionally be found in rabbits, guinea pigs, and other rodent species. Often, the infection is asymptomatic in species that are not the normal host. Clinical infection in the genital tract can lead to infertility (metritis and pyometra may occur), embryonic resorption, and reduction in litter size. Diagnosis can be challenging because culture is often negative, although serological testing is also available. Treatment is based often on known sensitivities of the organism because culture is difficult; fluoroquinolones and tetracyclines are effective and safe for most species (Morris 1995).

Listeria

Listeria monocytogenes is a Gram-positive bacterium that is implicated in cases of late term abortion (due to acute metritis and placentitis) in rabbits and chinchillas. It can cause sporadic deaths or limited epizootics in breeding establishments, leading to significant losses. Oral penicillins are the treatment of choice in many species, but these are categorically contraindicated in rabbits and rodents. Tetracyclines can be considered for treatment based on culture and sensitivity results.

Brucella

Brucella spp. have been implicated in cases of infertility in rabbits; however, rabbits and rodents are not natural hosts of these bacteria. Although infection and clinical signs related to the genital tract remain possible, clinically, this is uncommon. It has been suggested that rodents are a reservoir of Brucellosis for free ranging ungulates; however, many of the rodent species tested were not seropositive for this organism. There are no reports of Brucellosis in either the African pygmy hedgehog or the sugar glider in the literature.

Chlamydiaceae

Chlamydiaceae (*Chlamydophila caviae* and *Chlamydia muridarum*) may be transmitted venereally and can be a cause of metritis and infertility in many species of animal, including small mammals (Deeb et al. 1989). Chlamydial organisms do not damage tissues directly; rather they induce a damaging host response at the affected site, which leads to clinical signs. The zoonotic implications should be considered if Chlamydiaceae are found.

Viral

There are a wide range of viral diseases in small mammals, and several can directly or indirectly affect reproduction and fertility. Most studies on these pathogens have focused on their control in groups of animals, including susceptible strains, kept for breeding and used in biomedical research. When such pathogens are introduced into such naive populations, the acute effects of the resultant disease can be very significant. However, for pets with small numbers of immunocompetent animals that may have been exposed and recovered or when the disease remains in subclinical, the effects on reproduction may be far less obvious or even of limited significance.

Rat Parvovirus

Parvoviral infections have been recorded in several species of rodents (hamsters, rats, mice) as well as in rabbits. Parvoviruses in rodents tend to spare the gastrointestinal tract, and infection is likely to be subclinical in pet animals. In laboratory animals, fetal resorption and neonatal deformity and mortality may be seen, as well as periorchitis in infected males. There is no treatment available.

Sendai Virus

Species that are hosts of Sendai virus include mice, rats, hamsters, and guinea pigs, although chinchillas can be infected experimentally; gerbils appear to be resistant. Infection can be subclinical in rats, hamsters, guinea pigs, and rabbits. Susceptible animals show reduced breeding efficiency in addition to respiratory signs; there is no treatment.

Murine Mammary Tumour Virus

Murine mammary tumour virus (MMTV) is a retrovirus that affects mice specifically; the virus can be spread horizontally and vertically. In combination with exogenous factors such as chemical or physical carcinogens, MMTV can produce mammary tumours.

Mouse Hepatitis Virus

This is an RNA virus with numerous variants that are constantly mutating and evolving. There are two main types, polytrophic (respiratory) and enterotrophic (invades other tissues), that have distinctly different patterns of disease. The prevalence is high in both laboratory and wild mice.

Drugs Used to Manipulate the Reproductive Cycle in Small Mammals

As with other classes of drugs, those licensed for use in small mammals are small in number, but dosage information may be available in specialist formularies (Carpenter 2012).

Human Chorionic Gonadotrophin

Human chorionic gonadotrophin (hCG) is commonly used in exotic small mammal practice. Its physiological action is similar to luteinising hormone (LH) and causes follicles on the ovary to mature and ovulate, producing a corpus luteum. It stimulates testosterone secretion in males. Clinically, hCG is used to replace or supplement LH in cases of ovulation failure or delay (in rabbits), to induce lactation postpartum, to induce regression of ovarian cysts in guinea pigs, and to induce ovulation in ferrets with persistent oestrus. In males, hCG has been used to treat cryptorchidism, although this use in people is less supported (Thorsson et al. 2007), and to improve libido.

Deslorelin

A GnRH antagonist, Deslorelin desensitises GnRH receptors reducing the release of LH and FSH from the pituitary. It may be used for chemical castration (it reduces the production of both testosterone and sperm), which can last for up to 6 months. Deslorelin reliably blocks reproduction in both male and female ferrets and is an alternative to surgical neutering. It is also used as a treatment

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for adrenal disease in ferrets and rabbits and has also been used to treat ovarian cysts in guinea pigs; however, the mechanism of action is uncertain, and treatment failures have occurred. Deslorelin may also be used to treat oestrogen-induced mammary tumours in rats.

Aglepristone

A progesterone-receptor blocker, aglepristone is used for the termination of pregnancy in mammals. It has shown to be effective for this purpose in rabbits and rats, and although reproductive behaviour may be altered during the cycles immediately subsequent to treatment, there is no subsequent reduction in fertility. Aglepristone may also be used for the treatment of pyometra. Anecdotal evidence from its use in two hamsters and a guinea pig has shown that in combination with antibiosis and supportive care, it may cure pyometra with no reduction in subsequent fertility (Gogny & Fieni 2015).

Proligestone

A progestogen, whose action mimics cell metabolism changes caused by progesterone, clinically, proligestone has been used to delay oestrus in female ferrets as well as suppressing persistent oestrus in females out of season. Proligestone has also been used to treat pseudopregnancy in the rabbit. Although associated with fewer side effects than other progestogens, there is the potential for alterations to behaviour and temperament, as well as causing diabetes mellitus, cystic endometrial hyperplasia, and pyometra.

Cabergoline

Cabergoline is used to treat pseudopregnancy in many mammalian species. It is a prolactin inhibitor and has been used therapeutically to treat pituitary adenomas and mammary tumours in rats when there is a hyperprolactinaemia. Schoemaker (2017) has demonstrated that not all rats with mammary tumours and hyperprolactinaemia have pituitary adenomas, and the source of prolactin in these cases is obscure.

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41

Veterinary Control of Reproduction in Rodent Colonies

LUCY WHITFIELD, PHIL GIBBS AND TIM MORRIS

This chapter examines the breeding of small rodents as domestic, often children's, pets, rather than breeding for biomedical research purposes and complements Chapter 40 with a particular focus on practical aspects. The range of species discussed includes not only mice and rats but also gerbils, guinea pigs, and various species of dwarf and larger hamsters. Each species has their idiosyncrasies, but there are many general principles that apply to all of them. Pet rodents are usually bred for colour and coat type, so they may appear in a variety of forms. The main aim of this chapter is to help the veterinary student, and also practitioners new to this area, to optimise breeding and management by understanding the reproductive biology and the physical, social, and behavioural requirements of these species when bred as pets.

Most animals are sold shortly after weaning, although some more enlightened pet vending companies are starting to sell older, rescued, and exbreeding animals, particularly guinea pigs. This is to be commended, as these species may live for several years longer after the end of their breeding lifetime.

There is marked variation in the nature of pet breeding facilities, ranging from those units which have expanded gradually from hobbyists to a few units that are run by trained staff who may have degree-level qualifications in small animal husbandry and management. There are relatively few purpose-built, small rodent breeding facilities and, in general, little specialist advice available to the rodent breeder. Although a great deal is known about breeding the same species in the laboratory context, this does provide useful experience for the 'herd medicine' approach that is also necessary in pet breeding facilities (Suckow et al. 2012).

Breeding rodents commercially is about much more than just pairing animals and basic reproductive biology: their physical and behavioural needs must be taken into account and a great deal of planning and oversight are required to ensure that the number and type of animals bred matches the requirements of the customer. Animals that are stressed, either nutritionally or by their environment and care, will not breed successfully, and their psychological health may also suffer.

However, although the units are generally well organised, there are certain guiding principles that will help to ensure the good health and welfare of the animals who live there and also ensure that the animals that become our pets are of high quality and have good mental and physical health.

The facility must be organised with sufficient, trained, animal care staff to manage the animals and must have suitable accommodations for the species and ages of rodent animals that are kept. The correct determination of sex ('sexing') of all rodent stock is vital to ensure that suitable pairings are established which can mate and so that groups of animals are not left in mixed sexes until they are old enough to mate.

Criteria for Successful Breeding in Small Rodents

Species Biology and Characteristics

Key reproductive biological data for rodents and the other species are described in Chapter 40 and are summarised in Table 41.1.

Guinea Pig

With guinea pigs *(Cavia porcellus)* as with all species, it is important to minimise stress when catching and handling. Most of the guinea pigs' natural predators are airborne, so approaching their enclosure from above can provoke a stress response and panic if the animals are unused to regular handling. Spending plenty of time in the animal room is helpful to accustom adults and youngsters to human presence, and it also helps to speak quietly in the presence of the animals. Approach the animals from the side, in a lower posture rather than bending right over the pen. Providing a few tunnels or boxes for hiding can reduce anxiety in floor-housed guinea pigs. Whether catching them in either a cage or on a floor pen, this should be a swift smooth affair as guinea pigs are easily stressed by chasing and will become excited and difficult to handle. To pick up the guinea pig, place the thumb and forefinger around the neck with the palm across the shoulder and use the remaining fingers to assist the lifting; they should be supported at the rear with the other hand. It is better not to lift heavily pregnant sows, unless it is essential to examine them.

To sex the guinea pig, turn it over so that the body is supported on your wrist and look at the genital area; the shape varies between the sexes. If gentle pressure is applied in front of the genital region, the penis will be expressed in the male, whereas in the female the Y shape will always remain (Fig. 41.1).

It is essential to separate male and female youngsters at weaning, around 4 to 5 weeks of age, as guinea pigs can mate at 7 weeks. They should not be mated for the first time until they are 12 to 14 weeks old in order to allow the young animal to grow. Note though, that the female guinea pig should be mated before 6 months

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TABLE 41.1 Key reproductive biological data for rodents and other small mammals

Spp	Life span	Sexual maturity	Oestrus Cycle	Gestation	Pregnancy diagnosis	Postpartum oestrus	Litter size	Age at weaning
Guinea pig	5–8 years	4–6 weeks	Nonseasonally polyoestrus. 15–17 day cycle, oestrus 1–16 hours. Spontaneous ovulators.	59–72 days	Palpation from day 15, ultrasound from day 21. Radiography from day 42.	Yes, within hours of parturition. 60–80% pregnancy rate.	3–4	14–28 days
Chinchilla	15–20 years	4–5 months in females, 7–8 months in males	Seasonally polyoestrus (November to May). Spontaneous ovulators. Cycle length 30–50 days. Oestrus 2–5 days.	105–118 days	Palpation from day 50, ultrasound or radiographs from day 60.	Yes, 2–48 hours after parturition, then 35–48 days after parturition (postlactation oestrus).	1–3	8–10 weeks
Degu	6–8 years	12–16 weeks in females, 16 weeks in males	Seasonal breeders, but in wild only breed once a year. Spontaneous ovulators. Cycle duration 21 days, oestrus 3–5 days.	90 days	Palpation from day 40. Radiography from day 50. Size limits usefulness of ultrasound, gestational sacs visible from around day 30.		3–10	4–5 weeks
Rat	2–3 years	Female 65–110 days, Male 65–110 days	Nonseasonally polyoestrus. Spontaneous ovulation. Cycle 4–5 days, oestrus 9–20 hours.	21–23 days	Ultrasonography from day 10.	Yes. Pregnancy can result, but if still lactating delayed implantation may occur: 3–10 day increase in gestation period.	9–11	20–22 days
Mouse	2–3 years	Male 50 days, Female 50–60 days	Nonseasonally polyoestrus. Spontaneous ovulation. Cycle duration 4–5 days, oestrus 9–20 hours.	19–21 days	Size limits ease of ultrasonography. Accurate diagnosis ultrasonographically from day 9.5 reported.	Yes. If lactating then delayed implantation can occur. Increases gestation by 3–10 days. Post partum matings may be infertile.	8–11	18–21 days
Hamster	3–5 years	Female 42–70 days, Male 70–98 days	Nonseasonally polyoestrus BUT reduced fertility in winter months. Spontaneous ovulation. Cycle duration 4–5 days. Oestrus 15–16 hours.	15–16 days	No definitive information.	No. Usually an anovulatory cycle.	5–10	19–21 days

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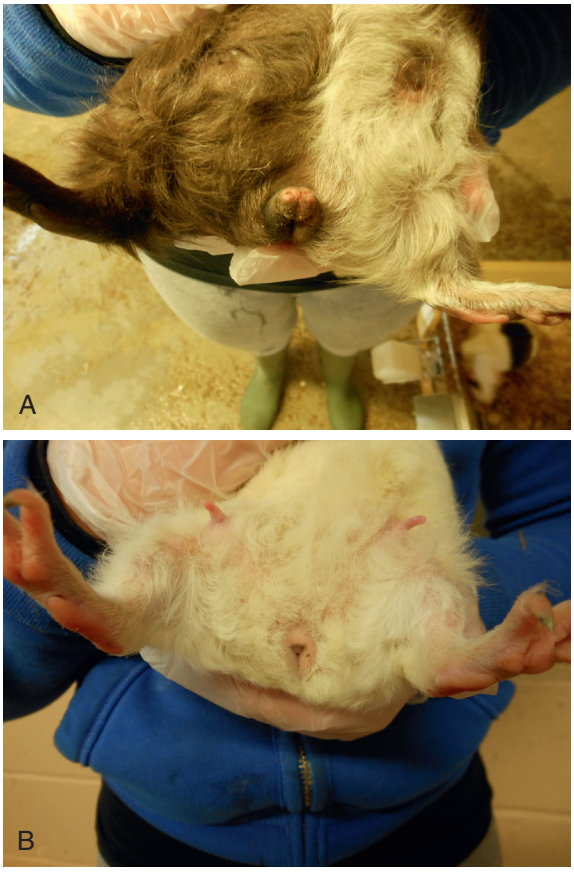
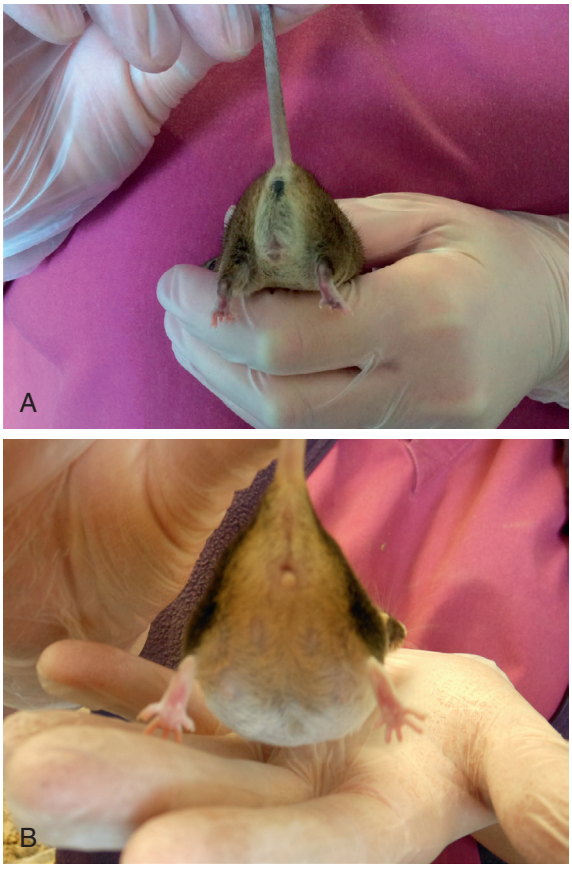
Gerbil	3–5 years	Female 65–85 days, Male 70–85 days	Nonseasonally polyoestrus. Spontaneous ovulation. Cycle duration 4–6 days, oestrus 12–18 hours.	23–26 days	No definitive information.	Yes. If lactating then delayed implantation can occur: increases gestation up to 48 days.	3–8	21–28 days
Rabbit	10–13 years	4–9 months, does generally reproductively mature earlier than bucks, but varies between breeds.	Seasonally polyoestrus: January to November. Spontaneous ovulation 9–13 hours after copulation. No defined oestrus cycle, but there are variations within their receptivity with peaks and troughs occurring every 4–6 days.	29–35 days	Visualisation of mating. Palpation at day 10–14. Ultrasound from day 14 onwards. Radiography from day 21 onwards.	Yes. Occurs within 9–13 hours after breeding. Pregnancy can occur, and proceed alongside lactation; however does may not be able to feed their offspring until normal weaning age, so supported feeding of kits and mother to reduce mortality may be required. Delayed implantation may also occur, leading to prolonged gestation.	6–8	Between 5 and 8 weeks of age
Ferret	8–10 years	Between 4–9 months, earlier in season when photoperiod is increasing and sufficiently long (12–14 hours).	Seasonally polyoestrus but between March and August approximately 50% of females remain in constant oestrus if not mated. Reflex ovulators.	39–42 days: 10–12 days preimplantation and active gestation of 30 days.	Abdominal palpation at day 12–14, ultrasonography day 12 onwards, radiography at day 30.	Varies depending on whether female still suckling young. If normal litter, then 14 days after weaning. Females with small litters may come into season earlier. Occurs within 9–17 days of parturition if kits are stillborn.	4–14	6–8 weeks
African Pygmy Hedgehog	2–3 years	8 weeks to 6 months in females, males closer to 6 months. Females may undergo pelvic symphyseal fusion by 12–18 months of age, potentially leading to dystocia if bred for the first time after this age.	Seasonally polyoestrus and probably spontaneous ovulators. Seasonality depends on weather conditions: hibernation occurs in cooler parts of range, whereas in warmer climates, African Pygmy hedgehogs will breed year round. Cycle duration 3–17 days	34–37 days. Delayed implantation may lead to prolonged apparent pregnancy.	Weight gain of 50g or more within a few weeks of breeding is suggestive. Visible abdominal swelling and mammary enlargement at day 30 onwards. Radiography from day 21. Abdominal palpation and ultrasonography may require anaesthesia and therefore the potential risk must be accounted for.		1–6	4–6 weeks
Sugar glider	6–8 years	8–12 months in females, 12–15 months in males	Seasonally polyoestrus. Primarily breed in winter and spring. Cycle is 29 days.	Gestation 15–17 days.	Difficult as conceptuses very small for ultrasound visualisation. Radiography and abdominal palpation are not appropriate.	Yes but if joeys in pouch then implantation will be delayed at blastocyst stage until joey leaves the pouch.	2–4	70 days



• **Fig. 41.1** Perineal area of male (A) and female (B) guinea pigs.



• **Fig. 41.2** Perineal area of male (A) and female (B) mice.

as after this time their pelvic bones can fuse, which possibly may interfere with natural birth (see Chapter 40).

Guinea pigs can be bred in pairs or trios in a correctly sized cage. However, in a commercial breeding environment, floor pens with one male and to up to 15 females are commonly used. This method is not as space-efficient as using cages but allows much more integration and natural behaviour for the guinea pig, as there is space and opportunity for more social and environmental enrichment than in a cage. Social housing also allows cooperation between females, in that they will help less experienced mothers and will allow other young inexperienced lactating females' young to suckle from them. Only one male should be kept with each harem group, as boars will fight in the presence of females.

Guinea pigs naturally form groups of five to six adult individuals, which will allow for social interaction among themselves and with neighbouring groups. Guinea pigs are generally very sociable, although it is not recommended to house females in groups larger than about 20 individuals; when larger numbers of animals are housed together, the groups tend to split into smaller social subunits (Kaiser et al. 2010). Guinea pigs form long-lasting bonds between each other, so moving around individual guinea pigs between pens can be very stressful. This has a bearing on breeding management, in which the number of females in a pen is reduced due to natural wastage; it is commercially advantageous to add more females to the pen in order to maximise occupancy rates and use of the floor space. 'Making up numbers' should therefore be done by combining existing groups of sows rather than adding separate individuals to the pen so that the existing social units are preserved.

Mouse

Mice (*Mus musculus domesticus*), similar to other small rodents, are prey species and careful handling is essential to reduce stress. Mice should not be caught and lifted by the tail, as this method has been shown to trigger a 'prey response' and result in high anxiety levels (Hurst & West 2010). Mice should rather be captured and lifted out of the cage in a 'tunnel' or in the cupped hands (Gouveia & Hurst 2013). The mouse can then be restrained for sexing purposes by holding the base of the tail, with the forelegs supported. Male mice can be differentiated by the greater distance of the genital opening from the anus, approximately double the distance of the female (Fig. 41.2). The scrotum can also be observed in mice of 3 weeks of age and older. An additional help in determining gender in young mice of 7 to 15 days of age is that hair does not develop around the teats of females so that it is easy to identify the spots (nipples) on the belly.

Mice should be separated at weaning (3 weeks of age) as they are capable of breeding at 6 weeks. However, it is wise to allow them to grow until at least 9 weeks before their first mating. They have an oestrous cycle length of 4 to 5 days and a gestation period of 21 days. Litter size may vary with mouse strain and the age of the female, but a good litter average is 6 to 12 pups. Commercially, a figure of 1.5 young per female per week is considered to be optimum.

Mice can be paired for life as the male will not be a threat to the pups, thus leaving the male in with the female allows for early postpartum mating, which minimises the length of time before the next litter. Mice can also be kept in trios or harems. Provided that

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there is sufficient cage space, trios are often preferred as this creates more young while still allowing for accurate breeding information on individual mice to be maintained.

Mice are usually allowed to have six or seven litters before being retired from breeding as fecundity in mice is reduced after approximately 8 months of age. Successful breeding is unlikely in individuals more than 12 months of age.

Rats

Rats (*Rattus norvegicus domesticus*) easily become accustomed to handling, and the rat can be picked up by placing the thumb and forefinger around the neck with the palm across the shoulders, using the remaining fingers to assist when lifting the animal. Care must be taken not to hold them too tightly as this will restrict breathing and cause the rat to struggle. Adult rats require support from the other hand when lifting, particularly if pregnant. Rats should not be picked up by the tail. When turning the rat over, the same anogenital distance differences described previously for the mouse can be observed; it is an easier task as structures are relatively larger (Fig. 41.3).

Rats are capable of breeding at 8 weeks of age, but it is again wise to allow further growth to occur in future breeders, with the time of first mating at 12 weeks old. They have an oestrous cycle of 5 days and a gestation length of 21 to 23 days. A good litter average is 6 to 12 pups, with a figure of 1.5 young produced per female per week being desirable. Rats are generally not kept for commercial breeding beyond 9 to 12 months of age as fertility and rearing success decline after this age.

Rats can be kept in pairs or trios; more females than this would require a very large cage for the young and would not be practical. As with mice, the male does not interfere with the young and can be seen interacting with young animals in play. Environmental enrichment is important in normal development of the pups, allowing them to experience a complex environment early in life. Exposure to ideal nesting materials at an early age ensures that future breeders will also be able to build good nests for rearing their own pups.

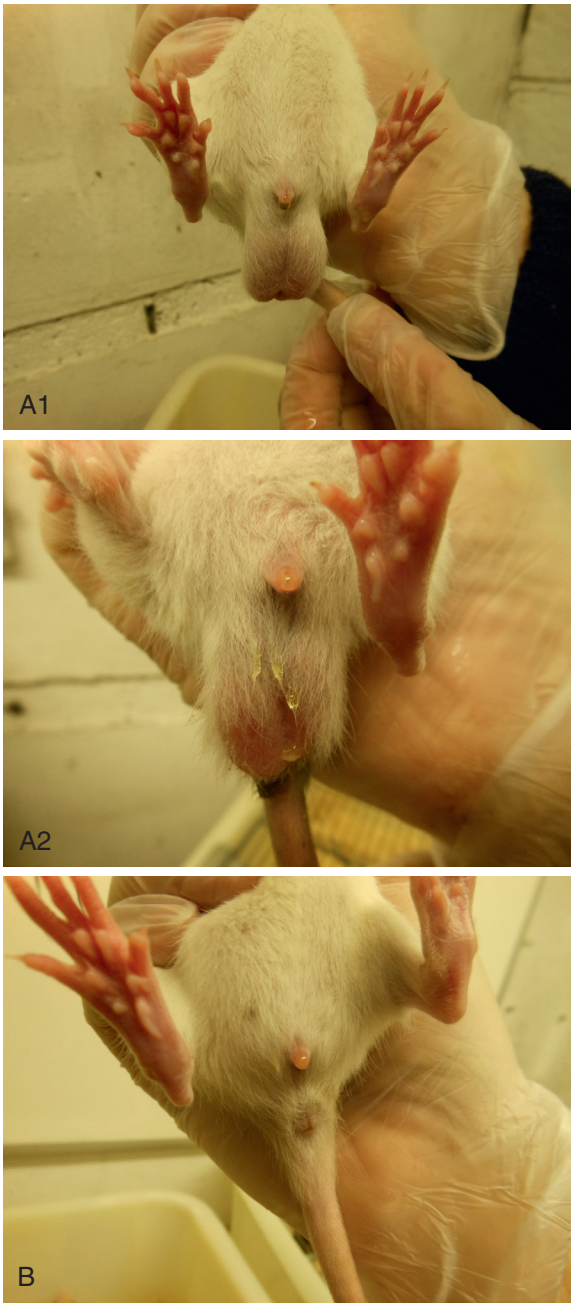
Syrian Hamster

The Syrian hamster (*Mesocricetus auratus*) can be picked up and then handled for sex determined in one of two ways. They can either be picked up by firmly but gently grasping them across the back or by grasping the scruff. Alternatively, they can be cupped using both your hands round the body, lifting the hamster up and turning it on its back by sliding the palms to the diagonal. A hamster may noisily indicate its displeasure at the intrusion, but no harm will come to either hamster or handler if no hesitation is shown on the handler's part. When the male hamster is turned on its dorsum the testes and scrotal sacs can be observed. However, in younger males, it is sometimes not obvious; in that case the comparison of the difference in the anal/genital distance between the male and the female is used. This can sometimes be a challenge especially if the hamster has long hair (Fig. 41.4).

Syrian hamsters are a solitary species so premating management is usually required. It is sometimes possible to keep Syrian hamsters in monogamous pairs if the male and female are paired at weaning and if there is enough space in the cage for them to seek solitude, although careful observation is crucial to identify signs of fighting. If fighting occurs, the male should be removed from the cage and generally cannot be reintroduced in the long term.

Traditional hand mating is successful, but it is a labour intensive method of breeding. Hamsters are mated soon after dark by placing the female in the cage of the male. The duration of the oestrous cycle is 4 days and thus the female will accept the male on only 1 day of the 4. If he is accepted by the female, the mating will be observed almost immediately, and the male can be removed quickly to avoid the possibility of fighting.

The third and most viable way of commercially breeding Syrian hamsters is to house a pair together for 6 days as this ensures the female will have ovulated at least once during this time. The pairing should be observed frequently to ensure there is no fighting. The male is then removed on Day 6, and the female is allowed to 'litter' and raise her young. The male can be used again to breed with other females, although it is prudent to allow a week of rest and recovery before the male is used again, so sufficient males must be present in the colony to allow for a week of mating and a week of rest. In practice, this means accommodating two males



• **Fig. 41.3** Perineal area of male (A) and female (B) rats.

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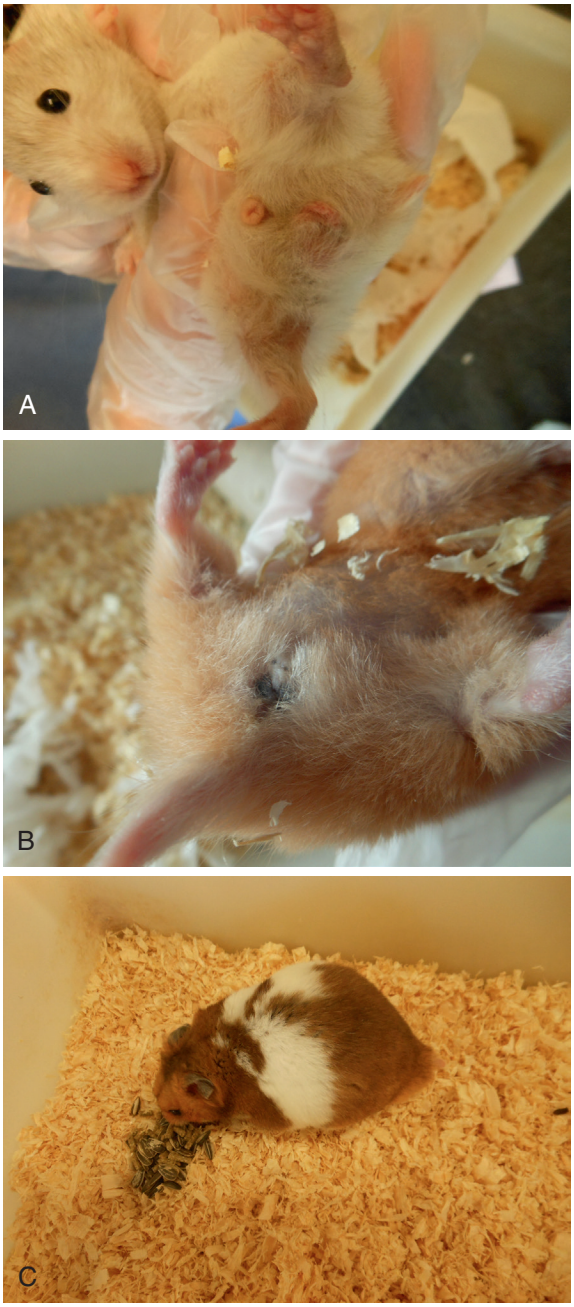


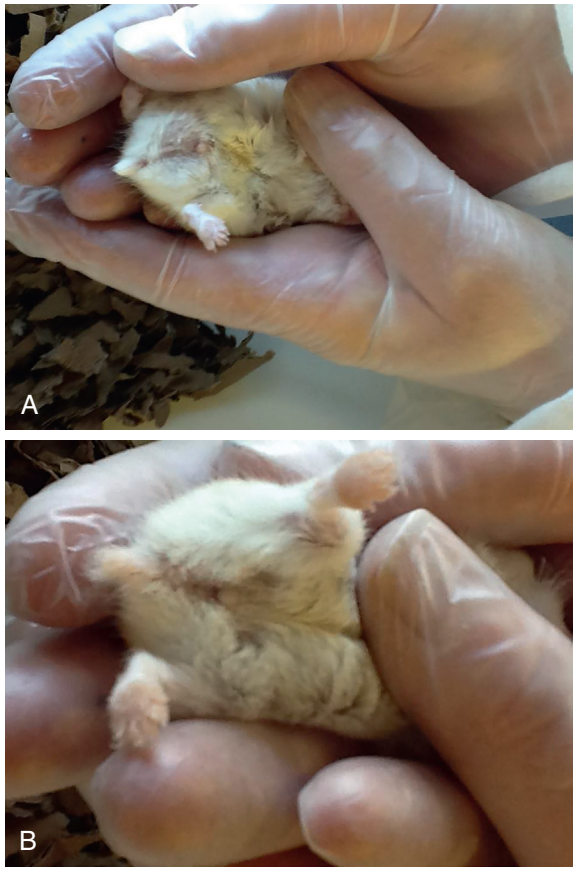
• **Fig. 41.4** Perineal area of male (A) and female (B) Syrian hamsters. (C) In some cases you are able to determine sex without the need to handle; note the obvious testicles.

per five females and rotating them over a 5-week cycle of mating, pregnancy, lactation, and resting.

The normal litter size in Syrian hamsters will be between 4 and 12, and a colony average of 6 or more is expected commercially.

Dwarf Hamsters

The most common species of Dwarf hamsters bred commercially are the Russian (*Phodopus sungorus*), Chinese (*Cricetulus griseus*), and Roborovski (*Phodopus roborovskii*). To pick up a dwarf hamster requires a speedy reaction, although the Chinese hamsters are a little less responsive to interferences. Place a cupped hand gently over the hamster, and taking the scruff between the thumb and forefinger, the hamster can be turned over to be sexed. As for the other small rodents, the anal–genital distance is the distinguishing difference, being greater in males than females. Comparing the sexes allows the handler to become proficient in observing the small difference in the distance and is essential for good colony management. The adult male Chinese hamster has easily identifiable testes (Fig. 41.5).



• **Fig. 41.5** Perineal area of male (A) and female (B) dwarf hamsters.

Monogamous pairing is the most satisfactory method of breeding dwarf hamsters, with first pairing at 12 weeks, allowing the young the correct amount of growing time. Social development is important in these species, and, similar to gerbils, dwarf hamster fathers, particularly the Russian, are interested in the preweaning young and can be observed interacting in a positive way with the young in the cage.

The species vary in the length of time from first pairing to breeding – Roborovski hamsters may take several months to produce their first litter, so the colony numbers should be adjusted to take account of the fact that up to 25% of the colony may not be breeding. This is particularly apparent in the winter months, especially when light and temperature may be lower. In contrast to the management advice for most rodents, it is recommended that Roborovski pairs are not paired before 6 months have elapsed as some do suddenly start breeding very productively even at this stage.

Mongolian Gerbils

Gerbils (*Meriones unguiculatus*) can be picked up by closing cupped hands around the animal, as tail capture is likely to be stressful and there is also a risk of a 'degloving' injury to the skin of the tail. Turning gerbils onto their dorsum causes anxiety, so they should be sexed by lifting the hind legs off the palm of the hand while supporting the front legs. The greater anal–genital distance and

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• **Fig. 41.6** Perineal area of male (A) and female (B) gerbils.

obvious scrotal sac in the males make sexing relatively straight forward (Fig. 41.6).

Gerbils naturally live in social groups consisting of the breeding adult pair and several generations of youngsters, which are usually inhibited from breeding by the dominant adults through suppression of sexual maturation in the females, although the testes and sexual characteristics of the male offspring continue to develop normally. Offspring should be separated from the parents by weaning at 3 to 4 weeks of age and kept in separate sex groups until mating.

Gerbils should be first mated at the relatively early age of 6 to 7 weeks, in order to minimise the chances of a nonreceptive female attacking the male, which often happens if individuals are paired when mature. Male gerbils are important in supporting breeding by assisting with nest building and taking care of pups by cleaning and warming them; this appears to stimulate their activity and development (Clark & Galef 2000). Breeding in monogamous pairs is the perceived wisdom in the breeding of gerbils, although breeding in trios is also successful if the male and both females are introduced early and at the same time. There should be sufficient cage space, and the use of trios maximises breeding production in a single cage. Whether pairs or trios are put together, they should be initially closely observed and separated if any aggression is seen as this can escalate, resulting in serious injury if left together. It is not known why previously compatible pairs or trios suddenly become destabilised, but if older pairs or trios lose their mates, it is unlikely that they will accept another individual (Swanson & Lockley 1977).

Gerbils will usually produce up to eight litters in a breeding lifetime, although individuals vary, and it is important to make regular assessment of breeding success.

Macro and Micro Environment

The macro environment should remain optimal at all times in order to ensure animals' comfort and to maximise breeding success. The animal holding rooms should remain within a 4°C range as variation is likely to stress the occupants; 17°C to 21°C is comfortable for both animals and technicians. The effect of higher or lower temperatures on individual animals is also considered in Chapter 40. Ventilation should be at a minimum of 10 complete air changes per hour in order to remove moisture, dust, and microorganisms without exposing any animals to direct draughts.

Lighting should be provided by full spectrum lights of the correct lux, and this should preferably be complemented with natural light, which is good for both animals and their carers. Many rodent species are nocturnal or crepuscular, so light levels lower than 300 lux allow the animal carers to make observations and carry out husbandry tasks while not disturbing the animals. Rodents should have the opportunity to 'escape the light' by hiding in nests or shelters such as tunnels or 'houses'. The light–dark cycle should remain constant throughout the year; 14 hours light to 10 hours dark is the author's preferred ratio. Shortening day length mimics autumn to rodents, and the drop in production would be marked. It is essential to check at intervals that the timing system for controlling the lights is functioning correctly as failure to turn on or off the lights at the required times can have profound effects on the animals' welfare and breeding success.

The micro environment for breeding rodents is equally important. The recommended correct cage and pen size for each of the species has always varied, the guidelines varying between licensing authorities and over time. Many commercial pet breeders use ex-laboratory caging, which no longer meets the EU specification for size. Space per se is not the only factor that affects whether or not the animal can meet its needs; proper animal care dictates that any breeding animal should have enough cage space to interact with or move away from their partner or youngsters and enough space to build a nest and raise a litter (Van de Weerd et al. 1997, Patterson-Kane et al. 2000, Young 2013). There must be enough space to have some form of environmental enrichment; small tubes or houses are a simple way to give the rodent another platform or hiding place, and swings promote physical activity and development for the youngsters (Fig. 41.7). Allowing the animals to carry out natural behaviours will safeguard their welfare; for example, rats investigate their environment by orienting on their hind legs and so require sufficient cage ceiling height to achieve this posture. Both rats and mice are agile climbers and benefit from being able to use the vertical dimension of their cages through ropes or ladders. This also allows them the opportunity to spend time away from their litters occasionally. Regular handling assists the animals to acclimate to human contact, ready for their pet homes.

Nest building is partly innate and partly a learned behaviour; thus rats or mice born in environments lacking a nest will not build such good quality nests themselves; thus provision of suitable soft, manipulable materials is essential. These may include cotton-based materials as well as paper towel, paper strands, paper 'wool', hemp strands, or hay. Providing a shelter is also helpful; these may be cardboard, plastic, or wooden and suitably sized for the animal and the cage. Breeding rodents should be disturbed as little as possible to reduce stress during late pregnancy and in the neonatal period. Small rodents use scent cues heavily, so cage cleaning can be very disruptive to the animals' social environment; moving the home nest to the new cage when cleaning out helps to provide a familiar environment for the occupants.

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• **Fig. 41.7** Examples of environmental enrichment.

• **Fig. 41.8** Tunnel to lead to nesting area.

Some species, such as gerbils, have a particular requirement for a long (> 25 cm) tunnel (Fig. 41.8) to lead to their nesting area, and providing this will increase reproductive success and reduce stereotypies. The correct nesting material gives them an opportunity to create a nest, which gives them a worthwhile activity and a comfortable area to be in and raise their young.

Sound or the lack of it plays an important part in maximising breeding production. A breeding area should be a quiet place with a minimum of sudden loud sound, particularly at the high end of the audible scale (ultrasound). Rodents are able to hear ultrasound, which humans cannot, so the squeaky extractor fan which we observe as being silent will upset them greatly. Similarly, vibrations travelling through walls or floor, such as caused by machinery or building works, are notorious for causing a sudden reduction in breeding activity. The use of radios by animal care staff is controversial as it adds noise that the animals cannot avoid. However, practical experience suggests that, provided that the noise is not loud, it becomes part of the regular routine, and, provided that it is switched off when activities cease, it does not generally cause detectable problems (Van Loo et al. 1994). It is also important that the human carers are relaxed and happy in their work, so if adding music to their routine facilitates this, then everyone benefits.

Nutrition

Breeding mothers are under considerable metabolic demand throughout their reproductive lives, and their offspring require excellent nutrition for their proper growth and development; therefore it is crucial that the nutrition is kept at a consistently high standard to provide the correct proportion of protein, carbohydrates, and fat. Rodent diets must also contain the correct balance of vitamins, amino acids, and minerals (Clarke et al. 1977, National Research Council 1995), further details are given in Chapter 40. Rodents do not generally overeat and are fed *ad lib* with a pelleted diet, although additional forage food such as sunflower seeds and cereal grains can be added on the cage/pen floor as an enrichment activity. Consistency and quality of the diet is crucial, and any reduction in diet standards leads to lower successful pregnancy rates, lower numbers born, and lower numbers of animals reared to weaning age. It is important to build a good relationship with the diet supplier or mill so that they understand the absolute need to provide a consistently high quality diet. It must be stored in a suitable cool, dry, and vermin-proof area, ensuring proper rotation of stock to prevent spoilage. In addition, guinea pigs require a good source of vitamin C; thus in addition to their pelleted diet, they should be provided daily with fresh vegetables and/or additional vitamin C in their drinking water.

Fresh water should always be provided to all animals at all times. Offering water in bottles is relatively labour intensive but does allow the technician to check water consumption as one means of assessing health daily.

Effective Management & Record Keeping

Good animal husbandry, in the form of regular cage changing, cleaning, and disinfecting, will minimise the risk of an outbreak of disease and reduce any preweaning losses. With large breeding colonies, this requires organisation to ensure every cage receives the correct level of attention while minimising labour requirements. Attention to detail is essential to ensure that stress is minimised during cage changing and pen cleaning; for example, transferring part of the animal's nest into the new cage helps ensure that there are familiar smells in the new cage and reduces the disruption of entering a new environment.

The requirements for different species vary, but in general, it is advised to leave a mother and young litter with as little disturbance as possible from the last week of pregnancy until the young have a covering of downy hair, at about 6 or 7 days. As long as the cage bedding remains dry, the hygiene risk is minimal and has to be balanced against the likelihood that disturbing the family by changing the home cage at this stage may lead to a mother abandoning or cannibalising her young.

A robust system of identifying animals or cages and record keeping is essential to a successful breeding programme. Basic information includes the number of breeding females, males, and

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• **Fig. 41.9** Example of identification and breeding details recorded on cage label.

future breeders present in the colony at any one time. The number of offspring weaned each week and 'in stock' as they grow should also be recorded on a weekly basis. Knowing the colony and the number of young required each week allows planning for the correct number of future breeders of the correct age to be available to replace the animals that need to be removed from the breeding system. Regular review meetings should be held between the unit manager and lead technicians from each species area or colony.

Inside the unit, cages should be clearly labelled (Fig. 41.9) with the identity of the animal(s) contained, including their parents, date of birth, and date that the pair was first prepared for breeding. The coat colour of the parents should also appear on the label so that any errors relating to incorrect identification can be promptly identified. For hand-mated rodents, such as hamsters, the cage card should include the identity of the stud male used in the mating so that outbreeding can be maximised. Stud males' cage cards should identify any unsuccessful matings in which pregnancy did not result so that subfertile males can be identified and removed after three unsuccessful pairings.

The breeding cage card should record the date of birth of each litter, plus the number of offspring born and weaned from the cage, including preweaning losses. By examining these records regularly, it is easy to identify any problems and take prompt remedial action. The first litter would usually be expected to be born 3 to 4 weeks after pairing (e.g., rats, mice, hamsters), and the female can be assessed for pregnancy after the first 2 to 3 weeks to confirm that all is well. Most rodents will mate postpartum, so there should be a regular production of litters. Dams or pairs who consistently fail to rear litters can also be easily identified and removed from the colony.

To maximise production, it is important to know when to stop breeding from a particular animal or pair. The litter size will naturally decline as the animal ages, so the age of the animal can be used as a guide; however, individual judgement on how each animal has and is performing will optimise a colony's breeding activity. An older mother may not produce so many young but may always rear all the young until weaning, so she may be worth retaining a little longer.

Some breeders find it helpful to arrange the colony by age, such that all animals of similar age are in a certain area of the room; therefore it is easier to identify those close to the end of their breeding lifetime or to give special attention to those littering for the first time. This is particularly helpful for managing species such as guinea pigs, which are bred in harems, so that the whole pen contains animals of a similar age. If younger and older animals are mixed together, it can be difficult to ascertain exactly what the true production level is, as the more fertile, younger dams can mask the presence of nonproductive animals.

The number of animals weaned should be recorded on a weekly basis in order to follow trends in production and to highlight any potential problems quickly. If individual animals are nonproductive, then the dam may be able to be remated with a different male (although this is not generally possible for gerbils). If the problem appears more widespread, then the primary cause should be investigated, encompassing management, diet, environment, and disease.

Typical Benchmarks for Pups Weaned by Species

The figures given here are typical production rates for each of the species, given as number of pups weaned per female per week. Note that the calculation should be made according to *all* the breeding females in the colony, not just those who are producing litters in a given week; otherwise, production rates will appear falsely elevated.

Mice:	1.5 per female per week
Rats:	1.5 per female per week
Hamsters:	6–7 per female per month
Gerbils:	2–3 per female per month
Guinea pigs:	1 per female per month

Troubleshooting: Problems With Breeding

Breeding failure is due either to failure to produce litters or to loss of pups before weaning. Although this may seem obvious, distinguishing which problem is occurring requires careful record keeping of the individual breeder's or pair's reproductive performance. Simply counting the number of pups weaned or sold is not sufficient.

For individual pet animals, it is easy to determine the reproductive success of the pair, but for a colony, analysis of the records and the production trends are required. The number of pups weaned is divided by the total number of breeding females in the colony, to give a 'number weaned per female per week' and should be calculated on a rolling basis. Downward trends or deviation of more than 10% from the weekly average should trigger investigation.

Trio or harem breeding systems can be monitored by recording the number of offspring per cage or per pen, again taking into account the number of females present. When multiple females are housed together, they should preferably be all of a similar age; otherwise, it is more difficult to determine which of the animals in the group may be nonproductive. This is particularly the case when older and younger animals are mixed together as the increased fecundity of the younger animals may partly hide that some females are not rearing young at all.

Investigating 'Failure to Litter'

Animal Causes

The first basic check is the gender of the pair. Missexing young rodents is not uncommon, and so it is worth double-checking who is in the cage. Check also for developmental defects, such as imperforate hymen in females or retained testicles in males. If the male is not a proven breeder (has produced litters with another female), then consider changing him for a new male. When hand-mating systems are used, then the male should be

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culled if none become pregnant after any of three separate matings (with different females).

Check the age of the breeding animals. Small rodents have a relatively short breeding life – for example, mice older than 7 months old are less productive, and mice over older than 12 months of age are unlikely to breed at all. Cystic endometrial hyperplasia is relatively common in older female mice, and these animals are unlikely to carry a pregnancy. Guinea pigs will breed for several years but may have fewer young and breed less regularly or with increased risk of dystocia when older than 4 years old.

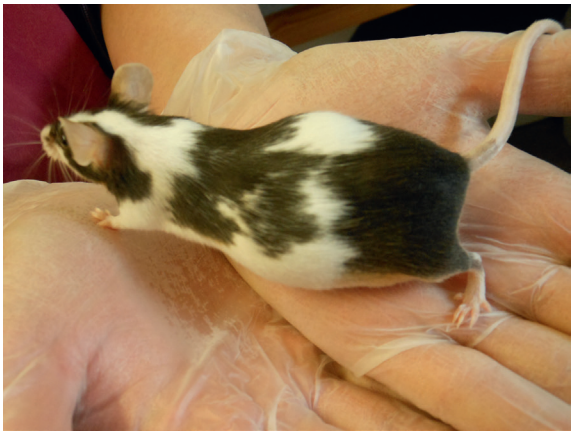
When setting up breeding pairs, it is usual to choose a male that is slightly older than the females. For small rodents, the strategy of choosing a male born a week or two before the females helps to prevent inbreeding in the colony, as the offspring must be from different parents.

Breeding for particular coat colour or other characteristics may result in inbreeding of a line, with depression in productivity. Certain matings may be incompatible and result in embryos that are not viable; therefore no pups are born. An example of this would be white-coated, long-haired hamsters, which cannot be bred together, as the resulting pups are not viable.

Nocturnal rodents generally mate during the nighttime dark period, so the event is not always observed. In rats and mice, the presence of a copulatory plug confirms that mating has taken place (although it does not confirm that pregnancy will result). The plug remains in the vagina of the female mouse overnight and can be seen the next morning, which helps to confirm the dates of mating and therefore the expected parturition date. The copulatory plug occurs in rats too, but tends to fall out, so confirmation of mating may be more difficult to detect. Plugs are not seen in guinea pigs or hamsters.

The female can be checked for evidence of pregnancy by observation for abdominal swelling at approximately halfway through the expected gestation period (Fig. 41.10). This may be more challenging in fat animals. It may be possible to gently palpate the fetus(es) in guinea pigs from an early stage and during the latter half of pregnancy in rats and mice. If available, ultrasound can be used to confirm pregnancy from approximately Day 8. The animal can be gently held for this procedure and sedation is not usually required.

If the time of mating (or setting up the pair) is known, then the cage should be carefully checked for the presence of pups at around the expected time of littering. Clear cages allow observation through the cage sides, or the cage can be gently pulled out of the rack in order to observe the nest without disturbing it. Some females (especially mice and hamsters) are very sensitive to disturbance, so there should be the minimum intervention needed to assess the health of the dam and litter. When neonatal deaths is suspected, an estimate of the litter size can be made on the day of parturition without interfering with the pups, then a more accurate count made 4 to 5 days later and again in subsequent days if required. This will distinguish whether a 'large' litter is born but then lost, or whether the litter is small from the start. Small spots of blood in the nesting material or cage may indicate that littering has taken place, even if pups have died or been cannibalised, and this will help to distinguish embryonic losses from postparturition problems.



• **Fig. 41.10** Abdominal swelling at approximately halfway through the expected gestation period in a mouse.

Management Causes

The circadian rhythm is very important in successful rearing and breeding of mice, and disturbances in the light cycle can have a significant effect on breeding success. Small rodents will breed with equal light/dark periods (i.e., 12 hours light, 12 hours dark), although conventionally, a slightly longer light period (e.g., 14 hours light, 10 hours dark) is suggested for optimising breeding. Disrupting the dark period by turning on lights (even briefly) can significantly disrupt the circadian pattern and disturb breeding. It is not uncommon that a lighting timer fails or that lights are accidentally left on at night, which may have a significant adverse effect on a colony's production.

As discussed elsewhere in this chapter, optimising the animal's environment is essential for their well being and for successful reproduction (Sherwin 2007). This includes both the quality of the animal's immediate enclosure and the wider macro environmental conditions, such as warmth, light, ventilation, and sound. Small rodents can hear ultrasound frequency ranges, as well as sounds audible to humans, so there may be disturbances occurring that are not immediately obvious to staff. Rodents recognise their human carers, and sudden changes in staff members (e.g., holiday periods) can affect breeding in sensitive animals (Sorge et al. 2014). Some technicians have better success than others with breeding colonies, so proper training, assessment of competence, and review of progress are important in ensuring a harmonious team.

The actual temperature (not just the heater's 'set point') should be checked, as variations in temperature can have a significant effect on breeding performance. A minimum/maximum thermometer should be used to record temperatures close to animal level and checked daily. For example, although guinea pigs will live at quite low ambient temperatures (and can even be kept out of doors in the UK), breeding and growth will be markedly reduced at temperatures lower than 15°C to 16°C.

Aged rodent colonies are less productive. (See also the section in this chapter regarding replacement of breeding animals.) A common cause of production decline is a failure to replace sufficient of the breeding animals, with the result that the colony ages, leading to reduced fecundity and rearing success. The breeding cycle and 'breeding lifetime' of small rodents is relatively short, so this problem can occur easily unless there is a consistently implemented system of replacement.

Investigating and Preventing Preweaning Losses

What is normal? Generally, small rodents rear their litters successfully, so preweaning losses are low. For example, typical preweaning mortality rates should be less than 5% for rats and mice and less

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than 15% for guinea pigs. Older mothers (see previous mention) may have higher loss rates. It is important to note whether the preweaning losses apply to one or two females/pairs or whether there is a more widespread issue in the colony.

Once a litter is noted, it should be recorded on the cage label or a mark placed on the cage; this provides a record for the breeder and also highlights that the cage should be subject to minimal disturbance. There are conflicting opinions concerning the age at which litters are counted; some prefer not to attempt counting until at least Day 5 after birth so as not to disturb the nest and to allow time for maternal bonding. However, counting and recording the number of pups born is part of good colony management, particularly when breeding a new line or species. Once it is clear that most of the pups born are still present at weaning, there is little need to count newborn pups routinely. Disturbing the litter may distress the mother and cause her to reject or even kill the pups.

If the pups do need to be handled, it is recommended that gloves are worn to prevent transfer of human scent onto the pups' skin and that hands are rubbed into the cage bedding to transfer the home scent onto the gloves. Pups should be returned to the nest as quickly as possible. The dams of some species, such as hamsters and rats, can be quite defensive of their litters, so removing the female from the cage before handling the pups may be advisable.

Strong maternal behaviour is essential for the well-being of pups, and this varies between individuals, strains, and parity. First-time mothers are more likely not to feed their pups and may cannibalise altricial pups at an early stage. As mentioned previously, minimal interference and providing a quiet environment with sufficient nesting material can promote good maternal behaviour. If the dam fails to rear two successive litters, then it is advisable to cull her. Breeding female replacements should be chosen from mothers with excellent maternal behaviours and rear larger litters. It is usual to choose replacement breeders from the third or fourth litters and avoid using animals from first or very late litters.

Neonatal pups of altricial species should lie together in the nest or shelter and not outside the nest. Absence of a nest (or poorly constructed nest) may indicate that the animal's environment is suboptimal and/or that the female is stressed or unwell and so would indicate investigation of the underlying cause. Disturbances, such as excessive noise, vibrations, or smell of predators may cause distress to the female. It is recommended that the home cage should not be changed for at least 7 days after a litter is born and that when the cage is cleaned, the entire nest is transferred to the new cage.

Appropriate parental behaviours (particularly maternal behaviours) are important for success in rearing young. Small rodents, such as mice, rats, and gerbils, build a secure nest during pregnancy, in which the young will be born and reared. When husbandry conditions are suboptimal, this may not occur and pups may be born outside the nest, with poor consequences for their survival. The quality of the nest can be assessed and scored (Deacon 2006); if poor nest building occurs more than very occasionally, the cause should be investigated as it may indicate poor environmental or husbandry conditions (Fig. 41.11).

It is relatively easy to determine whether or not the pups of small rodents are feeding by looking for the presence of a 'milk spot' in the stomach, which can be seen through the thin abdominal wall. Pups that are feeding normally will be pink, rounded out, and well hydrated, with translucent, clean skin. Lack of a milk spot may indicate lactation failure in the female. The dam will usually rapidly retrieve pups from outside the nest, and finding pups elsewhere in the cage may be a sign of poor maternal behaviour, disturbance of the cage, or illness in the pups.

• **Fig. 41.11** Mouse nest score 5 – full dome, with full height walls that enclose the nest.

If there is a problem with the birth mother, it may be possible to crossfoster neonatal pups onto dams with litters of similar age. Pups to be fostered should preferably be about a day older than the recipient's litter, and usually 2 to 3 pups can be added to an existing litter of up to 6 pups. Before adding the fostered pups, they should be gently rolled in the nest of the recipient and rubbed with one of the dam's own pups to help transfer scent.

Pups within the same litter normally grow at a similar rate; obvious disparity in size or body shape may be caused by congenital problems or competition for food. Once their eyes open, pups may be much more active and explore around the cage but should be found together in the nest when resting or feeding. Any pups that are obviously thin or smaller than their litter mates should be examined for abnormalities such as malocclusion teeth.

Placing a small amount of fresh soaked diet or gel on the cage floor each day may assist weaker pups to feed effectively. Commercially available jelly for human consumption can be used (it is preferable if it is made up to twice the recommended strength and fed 1–2 g per mouse pair or 5–10 g per rat). Ensure that any wet feeds are fed freshly every day, and any residue is removed in order to prevent spoilage. A small amount of seeds or wheat germ can also be added to the cage as a supplement – small rodents will forage for this foodstuff. These foods should be of food quality and appropriately stored to avoid possible microbial contamination.

Dystocia

Rats and Mice

Dystocia in most species of small rodents is uncommon and most litter without problems. If the dam is noted to be pregnant but passes the expected date of parturition without producing any pups, then she should be examined. Sometimes the abdomen takes on a 'triangular' appearance, with the base of the triangle close to the pelvis. The abdomen may be gently palpated for presence of retained pups. The small size of the vagina precludes digital manipulation of pups; thus Caesarean surgery is the only option if oxytocin (1 IU, given subcutaneously) is ineffective.

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Guinea Pigs

Dystocia is more common in guinea pigs due to the relatively large and well-developed pups. It may also result from metabolic disease (such as pregnancy toxaemia) or anatomical problems (such as a fused pubic symphysis). If the female is seen to strain for 20 to 30 minutes continuously or for a couple of hours intermittently without producing young, then dystocia should be suspected. The vagina can be carefully palpated with a finger to assess the degree of separation of the pubic symphysis, especially for primiparous sows. The bones should be separated by at least 10 mm in order to allow the pups to pass unimpeded. Provided that there is adequate separation, the sow can be given 1 to 2 IU of oxytocin subcutaneously, and allowed another 15 to 20 minutes for parturition to restart. If the pubic symphysis is fused or births do not follow the oxytocin, then a Caesarean surgery is indicated. More detail on specific issues with dystocia is provided in Chapter 40.

Laboratory Investigations of Breeding Failure

Laboratory investigations should be carried out if there are more than just occasional 'background' losses or breeding failure. The history and clinical signs will be helpful in suggesting appropriate tests; these would usually include postmortem examination of freshly dead or euthanised pups or adults and histopathological examination.

If an infectious cause is suspected, then blood samples can be taken and submitted for serology at least 2 weeks after the failure to litter, as this allows a suitable interval for seroconversion. Serology is helpful when an infectious (viral) cause is suspected; seroconversion and elevation of serum antibody levels occur approximately 14 days postinfection and may persist for several months, also enabling a retrospective diagnosis to be made. Small blood samples (20–50 µl) can be taken from the tail or saphenous vein of small rodents and blotted onto a laboratory card for serology. Alternatively, small blood samples may be collected and submitted in capillary or Eppendorf tubes.

Many laboratories can also carry out PCR testing on faecal samples or tissues to look for the pathogens themselves, which provides more immediate information in case of a suspected disease outbreak. Some viruses, e.g., parvovirus, are intermittently shed in faeces, so submission of tissues, such as mesenteric lymph nodes, may provide more accurate information about infection.

Fresh tissues may be swabbed for bacteriology, placed in formalin for histopathology, or frozen for molecular diagnostics, such as PCR testing. Animals that are found dead will usually be autolysed and unsuitable for histopathology, but PCR sampling is still possible, provided that care is taken to prevent contamination of the samples.

Suggested screening panel for reproductive problems in mice includes: mouse hepatitis virus; mouse cytomegalovirus; sendai virus; rotavirus; *Mycoplasma pulmonis; Citrobacter freundii;* cilia-associated respiratory (CAR) bacillus; *Pasteurella pneumotropica; Salmonella spp; Streptococcus pneumonia;* and *Clostridium piliforme (Tyzzers disease)*.

Suggested screening panel for reproductive problems in rats includes: parvoviruses (Toolan's H1, Kilham rat virus); sendai virus; rotavirus; *Mycoplasma pulmonis;* cilia-associated respiratory (CAR) bacillus; *Salmonella; Streptococcus pneumonia; Streptobacillus moniliformis;* and *Clostridium piliforme* (Tyzzer's disease).

Suggested screening panel for reproductive problems in hamsters and gerbils includes: LCMV; mouse parvovirus; and bacterial culture, including *Clostridium piliforme* (Tyzzer's disease).

Suggested screening panel for reproductive problems in guinea pigs includes: guinea pig cytomegalovirus; and bacteria, including *Clostridium piliforme* (Tyzzer's disease).

Additional information on specific diseases is provided in Chapter 40.

Maximising Production for Commercial Breeding

Forward Planning

A commercial breeding programme needs to produce the maximum amount of healthy stock from the minimum number of animals, thus saving space, labour, and supplies.

It is crucial that in a commercial environment, animals are produced that you actually need both for sale purpose and future breeding stock. Lacking the correct number means lost sales that cannot be retrieved at a later date. Breeding too many animals is a waste of animal lives, which is ethically unjustifiable, and has a commercial effect with ongoing animal husbandry costs.

A commercial breeder will have a core business and, consequently, a base figure of how many animals he or she needs to produce on a weekly basis, to satisfy customer requirements and put enough aside to grow as future breeding stock.

To illustrate how this works, imagine that you have a breeding colony of 200 gerbils. Assuming an economic breeding life of 8 months for the adult trios, approximately 12.5% of the colony, or 24 adults, should be replaced every month. When the gerbils are being bred as trios, this means saving 16 females and 8 males to replace those that retire each month. It is important to save future breeders every week and consistently through the year; otherwise, a decline in production may occur as the colony ages or after a sudden influx of new breeders. At busy times, it is tempting to sell all the animals produced, but this short-term action can have significant effects on later productivity.

In addition to maintaining the productive base colony, there are always trends of highs and lows in numbers sold throughout the year, such as the marked increase in guinea pig sales throughout the summer months, and a spike in small rodent sales in September as people return from their holidays and children go back to school. The period immediately after Christmas has recently become a busy time, as pet outlets prefer not to sell pets as Christmas presents, so sales in December are low.

Planning the number of animals that are required throughout the year needs forethought, similar to any farming enterprise. Before more animals can be added to the colony, there has to be a small increase in production to cover the additional future breeding stock that will be required. Assuming that the production level per animal remains constant, an increase in colony size of 20% will require 20% more breeding females, plus the corresponding increase in males depending on the particular breeding system that is used (e.g., trios, pairs, harem).

The first action is to calculate the date or period of time in which the increased production is required and then to work backwards, taking into account the biological characteristics of the species in terms of gestation and growth. To produce most small rodents, such as hamsters or rats, the time from mating until sale of the youngster comprises approximately 3 weeks' gestation, plus 3 weeks preweaning, plus 1 to 2 weeks before they are of sufficient size for sale, giving a total lead time of 7 to 8 weeks from mating to sale.

The time taken to produce adult breeders then needs to be considered, as these need to mature to the correct age for breeding

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before they can be included in the colony and begin mating – at approximately 8 to 12 weeks of age. The lead time between addition of new breeders to a colony and sale of the first pups is at least 19 weeks in advance. Where guinea pigs are concerned (gestation of 8–10 weeks), the lead-in period is nearer 6 months. It is therefore essential that plans are discussed and implemented well in advance so that production and sales of animals are well matched and under- or overbreeding are avoided for both economic and ethical reasons.

The Production Management Team

Successful rodent breeding on a commercial scale depends on the diligent input of a team, including the unit manager, technicians, nutritionist, and veterinary surgeon.

The animal carer/technician has a very significant effect on the success of breeding, and sudden changes in care staff can have an effect on breeding. The technician is seeing the individual animal and the colony every day, checking food and water intake, checking individual animals and their health status, recording all breeding activity on record cards, and recording daily events that can be quickly checked to see if anything about the colony could potentially be causing concern.

It is important that technicians are well trained in the basic biology and husbandry of the particular species with which they are working and also in identifying signs that may indicate ill health or distress in the animals of all ages. Courses run by the Institute of Animal Technology and some agricultural colleges provide helpful education and training and can lead to professional qualifications in the field.

Similarly, it is important to have veterinary input from a professional with a special interest or postgraduate qualifications in rodent medicine. As for farm animal 'herd health', this veterinary involvement should preferably take the form of strategic visits to review and advise on colony health and management rather than waiting until there is a problem or just seeing individual animals for clinical issues. Routine health screening of the colony is recommended in order to identify any organisms that may cause disease or production problems. It is essential at least to screen the colony for zoonotic organisms in order to ensure that the breeding animals do not present any risk to the unit's technicians and that the animals produced are fit and safe for sale to the public.

As for diagnostic workup, sampling need not require submitting whole animal carcasses to the laboratory as most can detect antibody in minimal volumes of blood using dried blood spots, and many organisms can be detected in faeces. A representative population of the colony should be tested, including older breeding animals and some weaned youngsters. This will ensure that both endemic and new organisms can be detected. The potential implications of any organism's presence can then be discussed with the attending veterinarian. Once an organism is known to be endemic in a population, it may not be necessary to continue testing for it in order to save costs if the assumption is that it is still present, and the decision is taken that it is not necessary to try and eradicate it.

Conclusion

Many factors need to be taken into consideration to successfully breed any species of rodent. Each species has their idiosyncrasies, but there are many general principles that apply to all of them. There is much experience in the breeding of laboratory rodents, and it is important to share the same aims of promoting excellent health and welfare for mutual benefit.

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42 Elephants

ANIL PUSHPAKUMARA, CHATCHOTE THITARAM AND JANINE L. BROWN

Elephants are the largest extant land mammal. They inhabit large areas of Asia and Africa and have maintained a tenuous relationship with man for millennia (Lair 1997). Important culturally and economically, elephants have been ubiquitously used as 'beasts of burden' and played important roles in war, agricultural colonisation, economic development, religious ceremonies, tourism, entertainment, and education (Sukumar 1989). Elephants also are important ambassadors for global conservation efforts; they are keystone species with a significant effect on ecosystems, umbrella species that indirectly protect other species in the same habitats, and flagship species that raise awareness for the support of broader conservation efforts. Once common throughout their native ranges, elephant numbers were severely depleted in the 20th century and continue to be threatened by poaching, human–elephant conflict, and habitat destruction (Lee & Graham 2006, Fernando et al. 2008, Stiles 2009, Wasser et al. 2009, Jackson 2013).

There are two genera of living elephants (Order *Proboscidea,* Family *Elephantidae*): Asian (*Elephas*) and African (*Loxodonta*). The African elephant (*Loxodonta africana*) is categorised as vulnerable by the International Union for Conservation of Nature (IUCN) and listed as two subspecies: savanna (*L. a. africana*) and forest (*L. a. cyclotis*). There are approximately 500,000 elephants free-ranging in 37 countries in Africa, about 25% of which are the forest subspecies. The Convention on International Trade in Endangered Species (CITES) has an agreement between governments to ensure that international trade in wild animal or plant specimens does not threaten their survival. In 1990 after nearly a decade in which African elephant numbers declined by almost 50%, the species was moved to Appendix I of CITES, which bans all international trade in ivory and ivory products. In 1997 some recovering populations (Namibia, South Africa, Zimbabwe, and Botswana) were moved to Appendix II, with strict limitations on trade in ivory. However, continued poaching and illegal trafficking in elephant products continue to decimate wild populations, particularly the forest species.

There are three subspecies of Asian elephant: Indian (*E. maximus indicus*), Sri Lankan (*E. m. maximus*), and Sumatran (*E. m. sumatranus*), with mitochondrial and microsatellite evidence that the Borneo elephant is a fourth subspecies (*E. m. borneensis*) (Fernando et al. 2003). Asian elephants are more at risk of extinction and listed as endangered by the IUCN, with less than 50,000 remaining in 13 range states, most (~60%) being in India. Compared with African elephants, the Asian elephant (*Elephas maximus*) has been listed in Appendix I since the treaty was enacted in 1975. However, Asian elephants are being killed not only for their ivory but for other body parts: skin, trunk, feet, or penis, all of which are in demand in the Chinese market.

If left undisturbed, both species breed well in the wild, but there is no guarantee that habitat protection or limiting human interference will occur fast enough to facilitate human–elephant coexistence in the long term. Breeding of elephants in captivity has been touted as a way to maintain valuable populations, but results have been inconsistent, and many are not self-sustaining. For the foreseeable future, species survival is likely to depend on effective conservation management, potentially supported by successful breeding *ex situ.*

Female Reproduction

Anatomy of the Reproductive Organs

The female elephant has the longest reproductive tract of any land mammal, approximately 3 m from vulva to ovary (Hildebrandt et al. 2000, 2003, 2006, 2011, 2012a) (Fig. 42.1); it has a number of unusual features: 1) a vestibule that averages 1.3 m in length and opens ventrally between the hind legs rather than below the anus; 2) a permanent hymenal membrane in nulliparous females that ruptures only at parturition; 3) a small vaginal os (4 mm x 2 mm in diameter); and 4) two blind pouches of similar size on either side of the vaginal opening (Fig. 42.2). The vagina can be up to 50 cm in length, with longitudinal folds. Mucus is produced during oestrus, and the mixture of expelled urine and mucus contains pheromones that attract bulls to oestrous females. The cervix is about 10 cm long and has a prominent cone-shaped opening. The uterus is bicornuate, with a short body (< 10 cm) and long uterine horns (> 60 cm). Implantation occurs in one uterine horn, although on the rare occasion when twins are conceived, fetuses are present in each horn. The oviducts are approximately 10 cm long, and the paired ovaries that are located caudal to the kidneys are proportionally small in comparison with body size (5.5–6.5 cm).

Puberty, Fertility, and Senescence

Females reach puberty between 10 to 14 years of age in the wild (Sukumar 1989, 2003) but can mature earlier in captivity (Glaeser et al. 2012), perhaps because of improved nutrition. It is not uncommon for Asian elephants to begin cyclical ovarian activity as young as 5 years of age, with the first pubertal rise in progestagens being quantitatively and qualitatively similar to subsequent cycles (Glaeser et al. 2012) (Fig. 42.3). It has been reported that wild African

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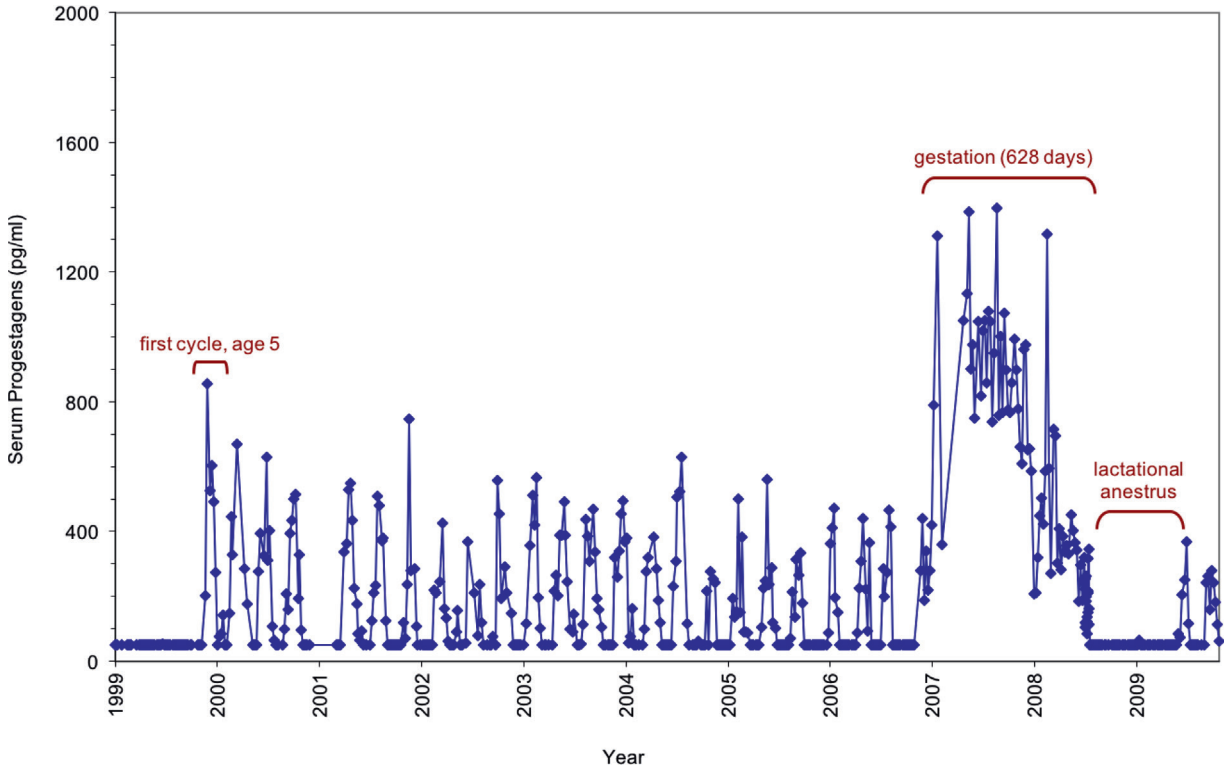
elephants can reproduce into their 60s, but for most, fertility ceases by the 50s (Lee et al. 2016). Similar large scale demographic data are limited for Asian elephants, although Sukumar (1989) has reported that wild Asian cows over 50 years of age also are capable of reproducing. A recent study conducted in the Udawalawe National Park in Sri Lanka, which has a relatively undisturbed population of over 1000 wild elephants, found the age at first calving to be about 13 years, with females reproducing until the age of 60 years (de Silva et al. 2013). At the Pinnawala Elephant Orphanage in Sri Lanka, females conceiving by 7.5 years of age are not unusual (Brown 2014). The youngest Asian elephant cow to give birth in the Myanmar Timber Enterprise was 5.3 years, and the oldest was 65 years (Hayward et al. 2014). For zoo Asian elephants in Europe, the earliest age at first reproduction was 5 years, with the mean age being 11 to 15 years (range 5–30 years), and the mean interbirth interval was 5.5 years (range 1.0–11.7 years). In US zoos, the youngest elephants to give birth were 5 and 10 years old and the oldest 41 and 32 years for Asian and African elephants, respectively. In wild African elephants, higher rates of reproduction have been observed in daughters with simultaneously reproductively active mothers, thus suggesting advantages from maternal (and grand maternal) coexistence (Lee et al. 2016). In Myanmar timber elephants, grandmothers exert a positive effect on grand calf survival, especially among young mothers, and the

• **Fig. 42.1** Schematic diagram of the female elephant reproductive tract: OV, ovaries; UT, uterus bicornis; CE, cervix; VA, vagina; VO, vaginal os with two bilateral blind pouches; VE, vestibule; UB, urinary bladder; U, urethra; PB, pelvic bone. (Adapted from Hildebrandt 2006.)

• **Fig. 42.2** Vaginal opening with two blind pouches in a nulliparous Asian female elephant as visualised by endoscopic examination.



• **Fig. 42.3** Longitudinal progestagen profile in a female Asian elephant, demonstrating initiation of puberty, continuous ovarian cyclicity, gestation, and lactational anoestrus. (Adapted from Glaeser et al. 2012 and used with permission.)

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physical presence of mothers increased rates of reproduction in their daughters (Lahdenperä et al. 2014).

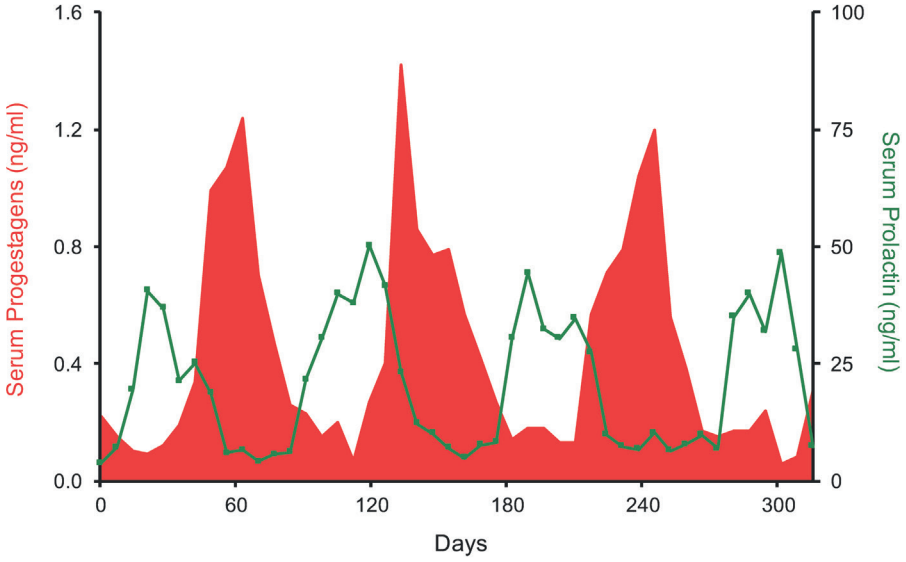
Maximum lifespan of wild African elephant females has been estimated to be 74 years, based on tooth wear (Lee et al. 2012), and approximately 80 years for Asian elephants (Lahdenperä et al. 2014). Evidence that elephants experience a true 'menopause' as in humans, with the cessation of ovarian cyclicity in midlife, is mixed. A large study of survival and reproduction of working elephants in Myanmar (> 1000 individuals) found reductions in fertility paralleled declines in survivor rate (Lahdenperä et al. 2014). By contrast, Lee et al (2016) reported a mean period of postfertile viability of 16 years in Amboseli African elephant females, which was based on an average fertility decline at 49 years and cohort longevity of 65 years. However less than 30% of that population survived after the period of fertility decline, suggesting only a small percentage exhibit this phenomenon. In that study, African elephant cows that commenced reproduction early had higher overall reproductive rates compared with those that first conceived at an older age. The early onset of reproduction did not affect survival nor did their calves experience reduced longevity (Lee et al. 2016). This is in contrast to the results from an extensive evaluation of semicaptive timber elephants in Myanmar (n = 8006), where an association between reproduction and adult survival was observed to be positive in early life but negative in later life (Robinson et al. 2012). It is not known if these contradictory data represent species differences, as evaluations of longevity, reproduction, and postfertile survival have only been conducted in a few populations.

The Ovarian Cycle

Elephants are polyoestrous and exhibit the longest spontaneous ovarian cycle of any mammal: 13 to 17 weeks in duration, with a 4- to 7-week follicular phase, and an 8- to 10-week luteal phase (Brown 2014). Based on hormonal and ultrasound assessments (Lueders et al. 2010, 2011, Brown 2014), a model of the ovarian cycle is presented in Fig. 42.4. Unlike most mammals, the major steroid produced by the corpus luteum (CL) is not progesterone, but 5α-reduced pregnanes (e.g., 5α-pregnane-3, 20-dione, 5α-pregnane-3-ol-20 one, 17α-hydroxyprogesterone), herein referred to as 'progestagens'. Oestradiol concentrations are low in the circulation due to rapid conjugation and urinary excretion (Czekala et al. 2003), and thus its measurement is of limited use for characterising follicular activity. Although elephants are easily trained for blood collection to track ovarian cycles, some hormones can be assessed noninvasively, e.g., progestagens in saliva, faeces and urine, and oestrogen-conjugates in urine (Wasser et al. 1996, Czekala et al. 2003).

A distinguishing feature of the elephant ovarian cycle is the 'double LH surge' that occurs in both species. Two precisely timed luteinising hormone (LH) surges occur 3 weeks (18–22 days) apart during the follicular phase of the cycle (see Fig. 42.4). These are referred to as LH1 (anovulatory) and LH2 (ovulatory) (Kapustin et al. 1996, Brown et al. 1999). The surges are quantitatively and qualitatively similar, although they are generally more distinct in Asian elephants. During the first wave, multiple follicles develop, but none ovulate (Hermes et al. 2000); instead, they luteinise and form accessory CLs (acCL) (Lueders et al. 2010). During the second wave, a new group of follicles develops, but only one becomes dominant, ovulates, and forms a single ovulatory CL (ovCL). All CLs persist throughout the follicular phase, with some of the larger ones remaining in subsequent luteal periods (Lueders et al. 2010). Formation of multiple CLs, in addition to the ovulatory CL, may be a mechanism to ensure there is sufficient luteal capacity for sustaining a long gestation should conception occur (Lueders et al. 2010).

As summarised by Brown (2014), inhibin secretion follows a distinct pattern in elephants (Brown et al. 1991, Kaewmanee et al. 2011a, Lueders et al. 2011, Yamamoto et al. 2012a). In other species, both small and large oestrogenic follicles can be significant sources of inhibin. However, in the elephant, circulating inhibin does not increase until after follicle luteinisation post LH1, which is about 9 days before LH2, suggesting it is the cells of luteinised follicles and acCLs that are the main source of inhibin. Inhibin also is increased during the luteal phase; immunohistochemical staining has localised inhibin subunits to the lutein cells of CLs, similar to that in primates (Yamoto et al. 1991, 1992). Follicle-stimulating



• **Fig. 42.4** Model of the elephant ovarian cycle, showing relationships between the secretion of oestradiol, progestagens, LH, and FSH, and development of follicles and corpora lutea (CL), both ovulatory (ovCL) and accessory (acCL). (Adapted from Brown 2014, Hildebrandt 2006, and Lueders et al. 2010.)

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hormone (FSH) secretion is inversely related to inhibin throughout the cycle in both elephant species (Brown et al. 1991, Brown et al. 1999, Kaewmanee et al. 2011a) (see Fig. 42.4). It is highest at the end of the luteal phase and through the first follicular wave when inhibin is low, then decreases to low concentrations as inhibin rises before LH2. The FSH pattern in elephants is more protracted, but similar to that of the horse, in which there are high concentrations at the end of the luteal phase that decline progressively towards ovulation (Brown et al. 1991, Brown et al. 1999, Kaewmanee et al. 2011a). Prolonged FSH stimulation may be necessary to induce the two successive follicular waves observed in the elephant, a feature that is absent in other studied mammals. In the face of declining FSH the transition of one follicle from an FSH-dependent to an independent state is likely to be required for dominant follicle selection, as described in other species.

Prolactin is increased during the follicular phase in African elephants and is believed to be important for follicular development (Yamamoto et al. 2010, Dow & Brown 2012), similar to that described for other species (Freeman et al. 2000, Frasor & Gibari 2003) (Fig. 42.5). By contrast, no such prolactin pattern is observed during the oestrous cycle in Asian elephants (Brown et al. 2004a). As discussed later, this species difference may explain why ovarian cycle aberrations related to abnormal prolactin secretion are common in African, but not Asian, elephants (Brown 2014). Recently, cortisol has been shown to exhibit a distinct pattern in both species, with concentrations being higher during the follicular phase (Fanson et al. 2014), the significance of which is unknown.

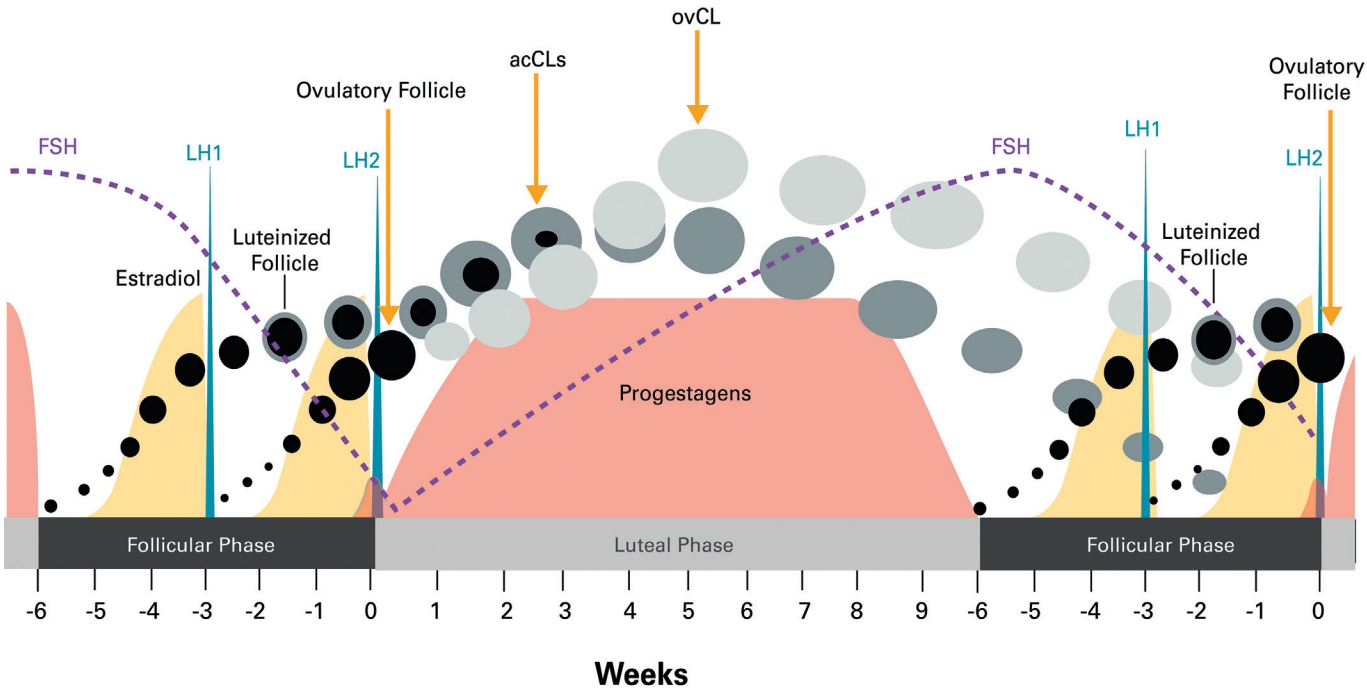
Both species advertise impending fertility through species-specific chemical signals. Female Asian elephants excrete a urinary signal, (*Z*)-7-dodecenyl acetate (*Z*7–12:Ac) that stimulates male breeding behaviour, the concentrations of which increase during the follicular phase and peak just before ovulation (Rasmussen et al. 1997, Rasmussen 2001). African elephants do not produce *Z*7-12:Ac, but excrete frontalin, exobrevicomin, endobrevicomin, (E,E)-alpha-farnesene, and (E)-beta-farnesene in urine, leading up to and during oestrus (Goodwin et al. 2006). Considering the long distances bulls travel in search of oestrous females in the wild, a prolonged pattern of pheromonal signalling would be a beneficial strategy for species survival.

Breeding and Conception

Copulation takes 2 to 3 minutes and may be repeated 4 to 6 hours later over a few days to up to a month. During copulation, the glans of the sigmoid-shaped penis is positioned at the end of the cranial vestibule (canal urogenital) and does not penetrate the vagina. The vaginal os is almost completely obstructed by an intact hymen (2 x 4 mm in diameter) in nulliparous females. The hymenal membrane remains intact during mating, rupturing only in conjunction with expulsion of the fetus. In general, semen is ejaculated at high pressure into the vagina through the vaginal os, explaining the observation of retrograde semen loss after mating and also the need for multiple matings by the male for successful impregnation. Elephants are one of the few species that have a ciliated endometrium (Mariappa 1986), presumably to facilitate sperm transport. After 8 weeks of gestation, a thick vaginal mucus plug is formed that provides a protective barrier against bacteria entering the uterus.

Pregnancy

Elephants have the longest gestation period of any mammal, lasting 20 to 23 months on average and typically giving birth to a single offspring; the birth weights of calves are 90 to 120 kg, with males generally being heavier. Lactational anoestrus lasts for 8 to 12 months, and the interbirth interval ranges between 4 and 6 years, depending on food resource availability. Calves nurse for up to 5 years, so females can be pregnant and lactating at the same time. Elephants are not obligatory seasonal breeders; however, conception rates often are higher during or shortly after peak rainfall in



• **Fig. 42.5** Longitudinal profiles showing the relationship between progestagen and prolactin secretion across three reproductive cycles in an African elephant cow.

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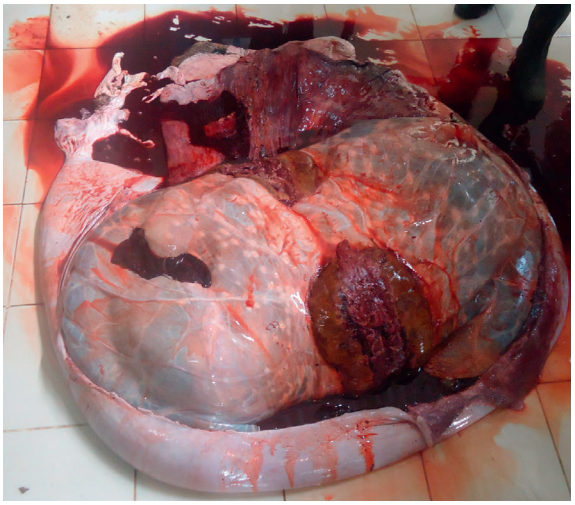
seasonal regions and can be negatively affected by drought (Sukumar 2003, Wittemyer et al. 2007). Elephants have reproduced into their 50s, although conception rates decline with age and the interbirth intervals lengthen.

Placentation and Implantation

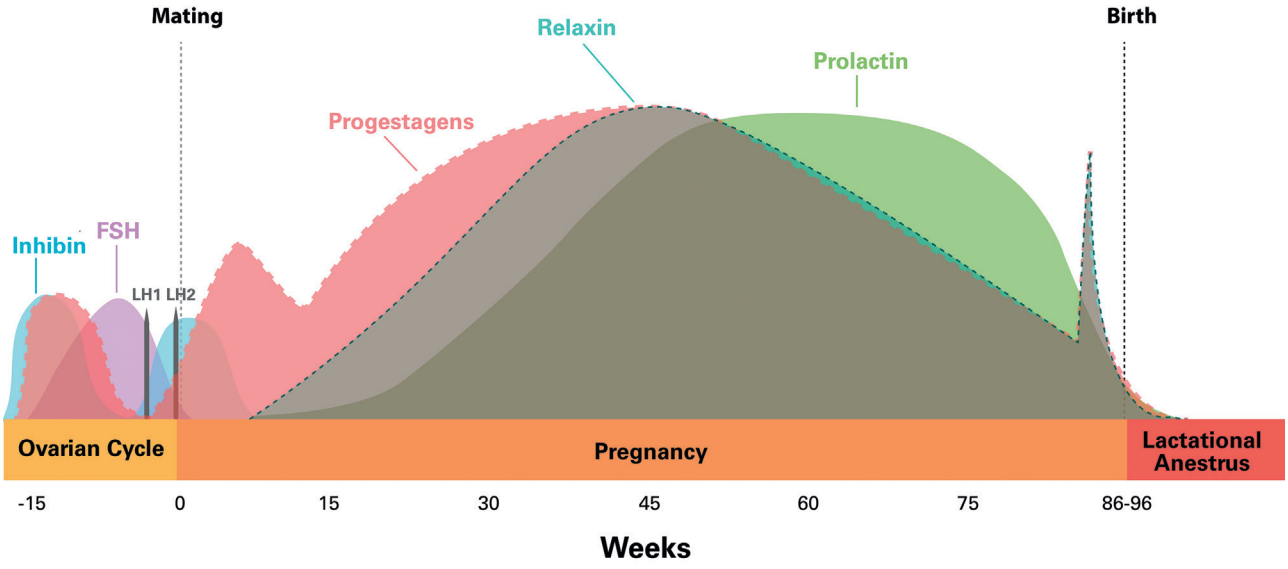
Implantation takes place in one of the uterine horns (approximately 50–70 cm in length) or in the 'pseudouterine body', as determined by postmortem analyses (Laws 1967) and ultrasonographic examinations (Hildebrandt 2006). Implantation is central and superficial, with a mesometrial orientation of the yolk sac and antimesometrial orientation of the embryo; it occurs during the second to third month of gestation (Drews et al. 2008). The placenta is zonarychorioallantoic, endotheliochorial, with some haemochorial regions (Allen 2006, Hildebrandt 2006) (Fig. 42.6). Longitudinal transrectal ultrasound has identified an initial period of comparatively slow embryonic development in both elephant species (Hildebrandt 2006, Drews et al. 2008). The embryonic vesicle is visible at approximately 8 weeks postconception, which is smaller (~ 10 mm) than that of other species at that stage (cattle, 40 mm; sheep, 70 mm; horse, 40 mm). Likewise, the time of implantation is estimated to be 20 days or less in human, dog, and sheep but 50 days or more in the elephant. The fetus then doubles in size between the fourth and fifth month and at 8 months drops into the abdominal cavity, so it is no longer is visible by transrectal ultrasound. Organogenesis is completed within about 110 to 120 days, when the end of the embryonic period is reached. Permanent uterine scars are formed by the invasive attachment of the placenta, which allows quantifying the number of pregnancies by ultrasound examination or at necropsy.



• **Fig. 42.6** Crosssection of the uterus of a full-term Asian elephant. Note the zonary type placenta.

Endocrinology of Pregnancy

A model of the hormonal relationships during gestation is presented in Fig. 42.7. Persistent acCLs are formed during successive oestrous cycles (Lueders et al. 2010, 2011), but no additional CLs are produced during gestation (Lueders et al. 2012). In both Asian and African elephants, acCLs and the ovCL begin to regress about 5 to 6 weeks after conception but then rebound and grow significantly larger than those in a nonpregnant luteal phase (Lueders et al. 2012), in conjunction with a marked secondary rise in progestagen concentrations (Meyer et al. 2004, Lueders et al. 2012) (see Figs 42.3 and 42.7). Based on histology and analysis of luteal progestagens, the CL of pregnancy is most steroidogenically active between 3 and 15 months of gestation. Inhibin is also elevated during the first 8 weeks, as in a normal luteal phase (Yamamoto et al. 2012a). However, in contrast to progestagens, which rebound to even higher concentrations, inhibin declines and remains at basal levels throughout gestation (see Fig. 42.7), suggesting that a role for inhibin in pregnancy is unlikely. After 5 to 7 months, both relaxin and prolactin markedly increase (Meyer et al. 2004, Yamamoto et al. 2012b) (see Fig. 42.7). Prolactin remains elevated throughout, whereas the relaxin profile is biphasic and concentrations start to decline after midgestation, with a secondary surge at parturition (Meyer et al. 2004). Pregnancy is typically confirmed through the longitudinal identification of serum, urine or faecal



• **Fig. 42.7** Model of the relationships between the secretion of progestagens, LH, FSH, prolactin, relaxin, and inhibin during gestation and a prior oestrous cycle in the female elephant. (Adapted from Meyer et al. 2004, Yamamoto et al. 2012a, and Lueders et al. 2012.)

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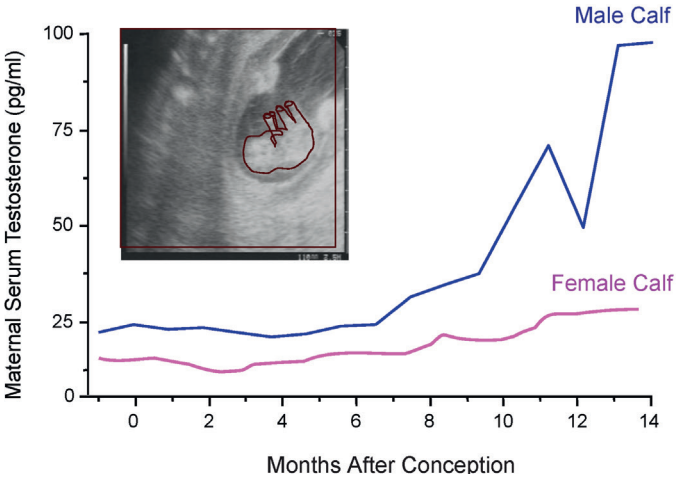
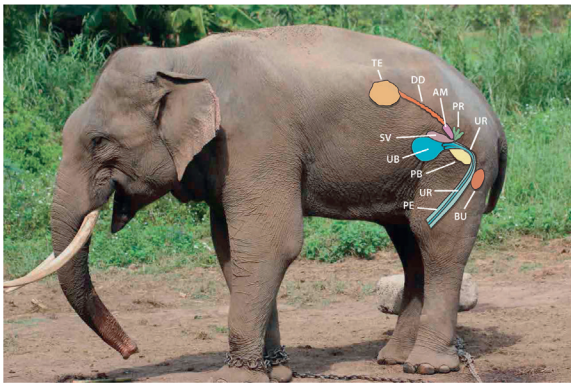


• **Fig. 42.8** Use of serum testosterone analyses in the maternal circulation to determine the sex of an Asian elephant calf.



• **Fig. 42.9.** Schematic diagram of the reproductive tract of the bull elephant: TE, testis; DD, ductus deferens; AM, ampulla; SV, seminal vesicle; PR, prostate; BU, bulbourethral gland; UB, urinary bladder; UR, urethra; PE, penis; PB, pelvic bone. (Adapted from Hildebrandt 2006.)

progestagens, and serum prolactin (prolactin cannot be assessed noninvasively in elephants) after 7 months of gestation. To date, there is no evidence of chorionic gonadotropin-like activity in serum or placental extracts.

Gestational hormone patterns differ somewhat between the Asian and African species. For example, progestagen concentrations are higher in African elephants during the first half of gestation but then decline to levels lower than those observed in Asian elephants, which exhibit a more biphasic pattern (Meyer et al. 2004). There is also a fetal gender effect on progestagen concentration patterns but only in Asian elephants, with females carrying male calves producing more progestagens than those carrying females (Meyer et al. 2004). Because of the internal gonads, it is not possible to sex an elephant fetus by ultrasound. However, sex determination is possible after about a year of gestation, with near 100% accuracy in Asian elephants by measuring circulating maternal testosterone; it is higher in females carrying a male calf (Duer et al. 2002, Brown et al. 2004b) (Fig. 42.8). This fetal-sexing technique is less accurate in African elephants. Parturition can be predicted on the basis of a rapid fall in progestagens about 2 to 5 days before birth (Brown & Lehnhardt 1995, Carden et al. 1998). Surges in cortisol often are observed in the last 2 weeks of gestation, with a significant surge at parturition, suggesting a role in the initiation of parturition (Meyer et al. 2004).

Parturition and Lactational Anoestrus

A review of elephant obstetrics has been published by Hermes et al. (2008). A week or more before parturition the female begins showing signs of discomfort, and in many females, passage of a vaginal mucus plug or mucoid discharge occurs in the days leading up to parturition. Once labour begins, the female becomes restless and changes positions often. Abdominal contractions increase in frequency but may not always be visually apparent. The cervical canal dilates due to pressure from the allantoic sac, followed by partial entry of the fetal legs into the vagina. A large bulge appears under the tail, initially consisting of the fluid-containing amniotic sac, but eventually the calf crests the caudal rim of the pelvis and slips out between the cow's hind legs; the second stage of parturition is relatively short, being 2 to 4 hours. The majority of calves are expelled in posterior longitudinal presentation. Newborn Asian elephant calves weigh 90 to 120 kg and are able to stand on their own within minutes of birth. The mother and other usually related females help guide the calf to the mammary glands almost immediately upon standing. All mothering or nonmaternal infant care is performed by any group member other than the mother in socially bonded groups of Asian and African elephant females; it is associated with better calf survival (Lee 1987, Lahdenperä et al. 2016). Milk composition varies throughout lactation, with fat and protein percentages increasing, and water and sugar percentages decreasing over the first year or two of a calf's life (McCullagh & Widdowson 1970, Parker et al. 2013).

The postpartum anestrous period generally lasts 12 to 24 months (e.g., Fig. 42.3). Retained fetal membranes, reduced milk production, death of a calf, or premature weaning can reduce the postpartum period to as little as 8 weeks (Brown 2000). The first luteal phase increase in progestagens after lactational anoestrus, when oestrous cycles resume, is similar to subsequent cycles (Glaeser et al. 2012) (see Fig. 42.3).

Male Reproduction

Anatomy of the Reproductive Organs

A schematic diagram of the bull elephant's reproductive tract is shown in Fig. 42.9. There are a number of anatomical differences between elephants and other terrestrial mammals (Hildebrandt et al. 1998, 2000b). The most notable are testes located intraabdominally and the absence of a pampiniform plexus. Thus spermatogenesis can take place at core body temperatures as opposed to being positioned outside the body in a scrotum to maintain a slightly lower temperature. The bull elephant also has no inguinal canal or cremaster muscle. The testes of bull elephants reach a maximum diameter of 15 to 23 cm and can weigh up to 3 kg each. They are located just behind and ventral to the kidneys; in a newborn calf, they are about 2 cm in diameter.

Spermatozoa produced in the seminiferous tubules travel to the epididymis through a rete testis system. In comparison to scrotal mammals, the elephant epididymis is not tightly attached to the testis. It is, however, still a long (150–200 cm) convoluted duct with three anatomically distinct regions (head, isthmus, and tail) where the sperm mature. The vas deferens is relatively short and coiled, with a terminal portion that widens to form the ampulla. The two ampullae are located dorsal to the neck of the bladder, where the bladder joins the urethra; they serve as organs for sperm storage before ejaculation and can be visualised by transrectal

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ultrasonography. The appearance and dimensions of the ampullae are a good indicator of reproductive status, and thereby serve as an indirect indication of testicular activity in bulls. A fully dilated and 'snowy' echogenic appearance of the ampullae indicates the presence of highly concentrated spermatozoa. Functional activity of the ampullae depends on the degree of testicular development and, in particular, circulating androgen concentrations.

The paired, cigar-shaped seminal vesicles are the largest accessory sex glands; they are located lateral to the respective ampullae and dorsolateral to the bladder. The maximum diameter of the seminal vesicles is about 10 cm, and they can reach a length of up to 45 cm. Each gland consists of outer muscular (5–6 mm) and inner mucosal layers easily visualised by ultrasonography, with a single cavity that can contain up to 400 mL of seminal fluid. Seminal vesicle fluid contains no sperm and is, therefore, less echogenic than that of the ampullae. This fluid (50–200 mL) forms the bulk of normal ejaculates; it is high in fructose, which serves as the main source of energy for the spermatozoa. Reproductively quiescent and adolescent bulls have empty seminal vesicles as assessed by ultrasound.

The prostate is a paired gland located above the pelvic urethra, caudal to the attachment of the ampullae. Each half of the prostate consists of three lobes, with the two halves connected by a bridge of tissue. In Asian elephants the three lobes of the prostate fuse into a globe-shaped structure, whereas in the African elephant there are clearly distinguished lobes with separate, irregularly shaped internal cavities. The prostate is the last accessory gland to develop during puberty, doing so only after the testes have reached a diameter of 7.5 to 10 cm. Thus there is a marked difference in the size and complexity of the prostate gland between adolescent and adult males, and an undeveloped prostate gland is difficult to distinguish from the connective tissues surrounding the urethra. The prostate gland of an adult Asian elephant (~2 cm) is generally smaller than that in African elephants (~5 cm).

The paired bulbourethral glands reach 10 to 12 cm in diameter in adults and are located immediately caudal to the root of the penis alongside the dorsolateral aspect of the urethra close to the ischial arch. Bulbourethral gland secretions are sticky and clear and are thought to be involved in cleansing and balancing the pH in the bull's urethra and lubrication of the vulva before intromission.

The penis of an adult bull elephant can measure up to 1 m in length and 16 to 20 cm in diameter and may weigh over 27 kg. It does not have a true prepuce, is highly vascularised, and, when erect, has an S-shaped upwards curve. The erect penis can be moved independently of the pelvis due to the presence of large paired elevator muscles. Thus there is very little pelvic thrusting during mating to achieve ejaculation. In a few individuals, a bulge can be seen under the tail formed by the penis and associated muscles, which becomes more prominent during musth but is not related to fertility.

Puberty and Social Behaviour

The commencement of spermatogenic activity (puberty) in young bull elephants occurs between 6 and 10 years of age. In captivity, successful matings have occurred in males as young as 6 years of age, but in the wild, mating is related to dominance status, and so most do not start breeding until they are 25 years of age or older (Sukumar 2003). Onset of puberty is affected by factors such as environment (for example, wild or captive), nutrition, and growth rate. A generally held misconception is that bull elephants are solitary, when in fact in the wild they usually travel in small, bachelor groups in search of oestrous females. Both captive and wild bulls form social hierarchies that are influenced by age, size, and musth status.

Sperm Characteristics and Semen Cryopreservation

Sperm Morphology

Elephant spermatozoa, as in other mammals, consist of a head and flagellum (tail) that are joined together by a connecting piece. It contains the acrosome, a DNA-filled nucleus, some cytoskeletal structures, and a small amount of cytoplasm. The head is spatulate-shaped and slightly smaller in African than Asian elephants, with lengths and widths of 6 µm and 2.5 to 3 µm, and 7 to 8 µm and 4 to 4.5 µm, respectively (Johnson & Buss 1967, Jainudeen et al. 1971, Heath et al. 1983, Kitiyanant et al. 2000).

The acrosome is a Golgi-derived vesicular sac, located on the anterior portion of the sperm head, which covers the nucleus. It contains hydrolytic enzymes, including glycohydrolases and proteases, that are released after sperm–oocyte binding. The process of exocytosis of the acrosome, called the acrosome reaction, is the final step before fertilisation and facilitates oocyte penetration for successful fertilisation (Tulsiani et al. 1998, Abou-Haila & Tulsiani 2000). Therefore a functional and structurally intact acrosome is essential for successful sperm–oocyte binding and penetration during fertilisation (Tulsiani et al. 1998, Abou-Haila & Tulsiani 2000). In elephants, vasculation between the outer acrosomal membrane and the overlying plasma membrane, which causes leakage of acrosomal contents, is observed during the acrosome reaction (Kitiyanant et al. 2000). The presence of apical vesicles and typical membrane vesiculation in the apical portion of the sperm head but not in the equatorial segments is the earliest sign of the true acrosome reaction. By contrast the acrosome reaction induced in dead spermatozoa is characterised by a random breakdown of plasma and outer acrosomal membranes. Increased cyclic nucleotide levels are observed in acrosome-reacted elephant sperm (Kitiyanant et al. 2000). The acrosome reaction is irreversible, and any damage or premature reaction of the acrosome can result in the permanent loss of sperm function and fertilising capabilities (Senger 2003). Mammalian spermatozoa often undergo spontaneous acrosome reactions *in vitro* (Yoshida et al. 2008) in the presence of unfavourable extracellular conditions, such as dramatic changes in temperature, osmotic conditions, or suboptimal storage media (Medeiros et al. 2002).

The sperm flagellum consists of three segments: middle piece, principal piece, and end piece. The middle piece is tightly wrapped with a mitochondrial sheath, which provides energy to the flagellum for cellular propulsion and motility (Hammerstedt et al. 1990). The midpiece is 10.5 to 11.1 µm long in Asian and 8.6 µm long in African elephants (Jainudeen et al. 1971). The flagellum, including the principal and end pieces, is 40.5 µm and 47 µm in length in Asian and African elephants, respectively. Total sperm length is 59.6 to 64.0 µm in Asian elephants and 61.6 µm in African elephants (Johnson & Buss 1967, Jainudeen et al. 1971).

Semen Collection and Evaluation

Normal ejaculates range between 50 and 200 mL in volume and are white in colour, although yellow, orange, and brown colour variants can occur. The earliest study on semen characteristics in elephants was performed with samples obtained from the urogenital tract of an Asian elephant immediately after copulation (Landowski & Gill 1964). A later study described seminal characteristics of

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a male Asian bull collected passively after being stimulated by a female in oestrus (Jainudeen et al. 1971). Several investigators have used electroejaculation in standing bulls under sedation or lying lateral under general anaesthesia (Jones 1973, Howard et al. 1984, Schmidt 1993); the latter is particularly applicable to studies of wild bulls.

Today, manual rectal massage is the most common method used to collect sperm-rich samples from captive bulls. It was first described by Heath et al. (1983) and subsequently by Price et al. (1986), who used either an equine artificial vagina, or a condom made from a rectal palpation sleeve, respectively. As described later in this chapter, the quality of semen collected by transrectal massage is often highly variable within and among individuals (Schmitt & Hildebrandt 1998). Manual rectal massage, after combined chemical (xylazine hydrochloride and butorphanol) and physical restraint, was shown to be effective in collecting semen from an Asian bull elephant (Portas et al. 2007); this offers a potential method for collecting semen from captive bulls that are difficult to handle directly, similar to those in musth.

Musth

Mature bull elephants exhibit a condition known as 'musth', which is characterised by heightened sexual and aggressive behaviour, temporal gland secretions (TGS) and swelling, and urine dribbling (UD) (Jainudeen et al. 1971, 1972, Ganswindt et al. 2002, 2005, Brown et al. 2007) (Fig. 42.10). The word is derived from the Persian 'mast', which means 'intoxicated'. Musth in most bulls occurs on an annual cycle that is not tied to a specific season of the year. Most healthy adult bulls exhibit musth at a predictable time each year, although the timing can vary between individuals (Lincoln & Ratnasooriya 1996, Hall-Martin & Van der Walt 1984). The heightened aggressive behaviour can last for as little as a few days to several months, and most elephant handlers believe that musth is a sign of good health. Musth is related to age, nutrition, and social status, with older, more healthy and dominant bulls exhibiting stronger musth signals (Jainudeen et al. 1972, Niemuller & Liptrap 1991, Lincoln & Ratnasooriya 1996, Hall-Martin & Van der Walt 1984). One study showed that when a younger bull entered musth, the mature dominant bull experienced more days in musth, had higher testosterone concentrations, and an increased intensity of musth behaviour (Duer et al. 2016).

• **Fig. 42.10** Musth in wild bull elephant in Minneriya National Park in Sri Lanka. Note the profuse TG secretion and semierect penis. (Photo courtesy of Dr. Oswin Perera.)

Bull elephants advertise their musth status through a variety of chemicals present in TGS and urine. In young Asian bulls younger than 15 years of age, TGS has a sweet odour due to the presence of acetates, an alcohol (3-hexen-2-ol) that smells of leaves, and pleasant smelling ketones (acetophenone and 2-heptanone) (Rasmussen et al. 2002, Riddle et al. 2006), known as honey or 'modamusth'; the latter is associated with behaviours that are more erratic and unpredictable. In addition, it is also of a shorter duration and associated with comparatively lower androgen levels. Modamusth has not been identified in juvenile African bulls, which may be a species difference. As Asian bulls age, the musth has a stronger odour due to the presence of carboxylic acids and frontalin, a chemical signal found in insects. In older Asian and African elephant bulls, musth urine contains higher levels of alkan-2-ones, alkan-2-ols, and some aromatic compounds compared with the urine of females and nonmusth males (Rasmussen et al. 2002, Riddle et al. 2006, Goodwin et al. 2012). Elephants have well-developed primary and secondary (vomeronasal) olfactory systems (Lazar et al. 2004), and both males and females respond to these chemicals. Oestrous females seek out musth bulls, whereas subadult and nonmusth males avoid them (Riddle et al. 2006, Goodwin et al. 2012). Ultimately, the state of musth confers an advantage to adult bulls, which gain more access to oestrous females, and experience a higher paternity success (Hollister-Smith et al. 2007); it is not necessary for a male to be in musth to impregnate a female.

There are distinct characteristics of the different musth phases. Rajaram (2006) described five stages of musth: premusth, early musth, midmusth, postmusth, and nonmusth. In premusth, the temporal glands begin to swell, and some volatile compounds are secreted; the elephant will often touch this area with his trunk. Appetite is reduced, and the bull will display frequent penile erections and masturbation. Testosterone levels are slightly elevated, about two to three times baseline. The early- to midmusth stages are characterised by more TGS, which becomes odourous as the period progresses. During full musth, temporal glands secrete a thick tar-like fluid, the bull becomes even more aggressive, is unable to extrude his penis, and dribbles urine continuously, which often leads to a greenish-white discolouration of the orifice. Captive males become disobedient, unpredictable, and highly aggressive in conjunction with a significant increase (10 to 20 times baseline) in circulating androgens (Ganswindt et al. 2005, Brown et al. 2007, Yon et al. 2008). It has been suggested that UD is a better indicator of musth than TGS (Ganswindt et al. 2005), at least in African bulls. In Asian bulls, aggression is associated more with TGS (Duer et al. 2016). These signs decrease during postmusth and disappear during the nonmusth period.

Male Endocrinology

As reviewed by Brown (2014), the endocrine control of reproduction in male elephants is similar to other mammals and includes increased secretion of GnRH from the hypothalamus, FSH and LH from the anterior pituitary gland, and the secretion of androgens (mainly testosterone) from the Leydig cells of the testes. Androgens are responsible for the development of secondary sexual characteristics and sex drive in male elephants, as in most other domestic animals. The secretion of androgens is under the direct control of LH; each pulse of LH from the pituitary gland gives rise to an associated elevation in circulating testosterone. Testosterone production in elephants is related not only to age but also to social rank, dominance, and the state of musth.

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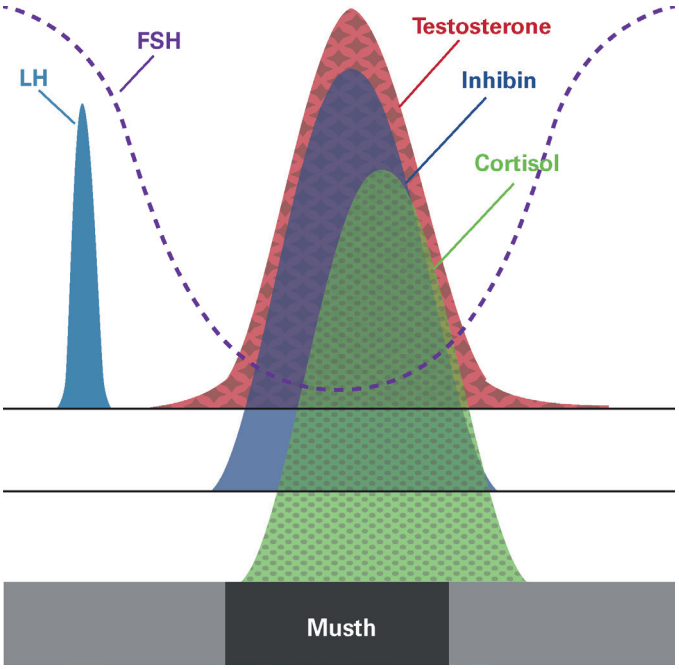
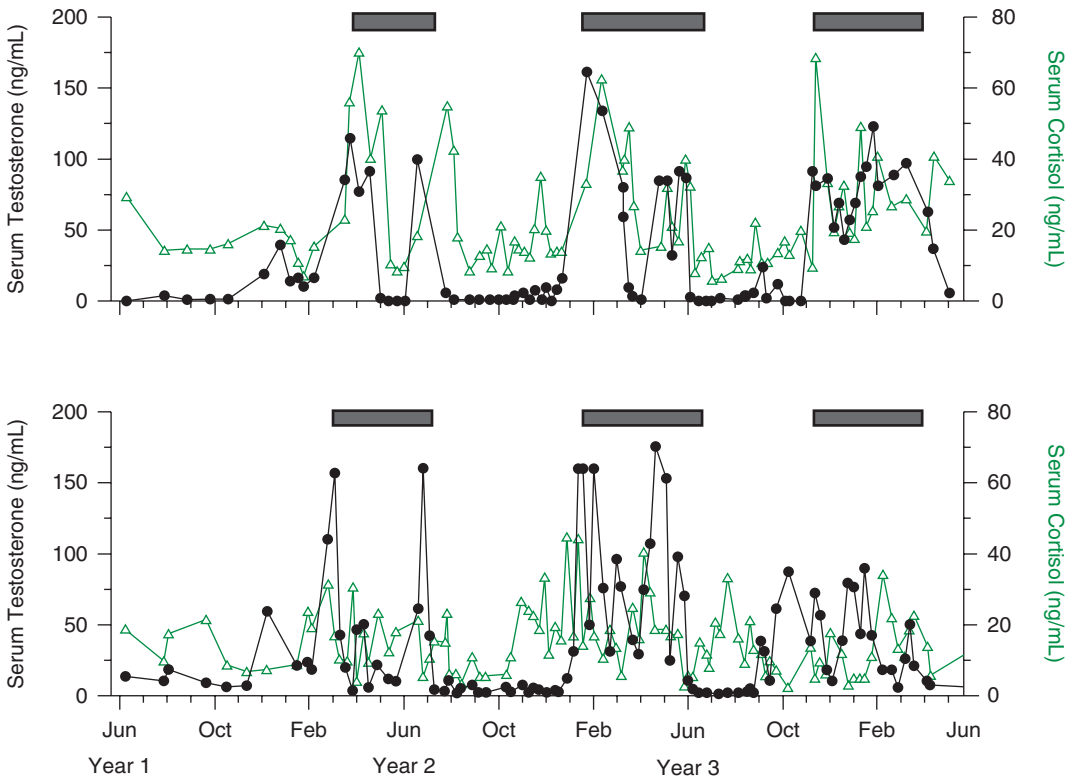
Musth is associated with a number of endocrine changes as shown in Fig. 42.11. Circulating cortisol is often increased during musth, at least in captive bulls, and is positively correlated with serum testosterone (Brown et al. 2007, Yon et al. 2007) (Figs 42.12–42.14). Serum gonadotropin patterns are not always consistent, but in many bulls, LH is elevated for several weeks about a month before musth begins, whereas FSH concentrations are higher during the premusth period and decline during musth (Kaewmanee et al. 2011b). FSH in elephant bulls is inversely related to inhibin, which in turn correlates positively with testosterone (Kaewmanee et al. 2011b). Another hormone produced by Sertoli cells under FSH control is AMH, which can be used as a marker of FSH and testicular function. AMH is higher in prepubertal compared with postpubertal elephant bulls; however, there are no differences in concentrations between musth and nonmusth males (Dow et al. 2011b).

Examples of normal annual musth cycles associated with elevated testosterone and the positive relationship between testosterone and cortisol are shown in Fig. 42.12. However, many bulls, especially in zoos, exhibit erratic musth periods, with multiple episodes a year (see Fig. 42.13) or are in a near state of constant musth (see Fig. 42.14). In terms of age, musth does not occur in wild bulls until after they reach their mid-20s, and bouts tend to be short and sporadic until the mid-30s (Poole 1989, 1994). However, in captivity, musth has been observed in bulls as young as 7 years (Cooper et al. 1990, Niemuller & Liptrap 1991, Ganswindt et al. 2005a). Fig. 42.14 shows the gradual change in endocrine patterns in a maturing zoo Asian elephant. Both serum testosterone and cortisol concentrations increased both overall and in peak concentrations, leading up to the first period of musth symptoms at 12



• **Fig. 42.11** Model of the hormonal relationships among testosterone, LH, cortisol, and inhibin in association with musth in bull elephants. (Adapted from Brown et al. 2007 and Kaewmanee et al. 2011b.)



• **Fig. 42.12** Individual profiles of serum cortisol and testosterone in two adult Asian elephant bulls demonstrating annual musth cycles. Musth periods are indicated by the black bars. (Adapted from Brown et al. 2007.)

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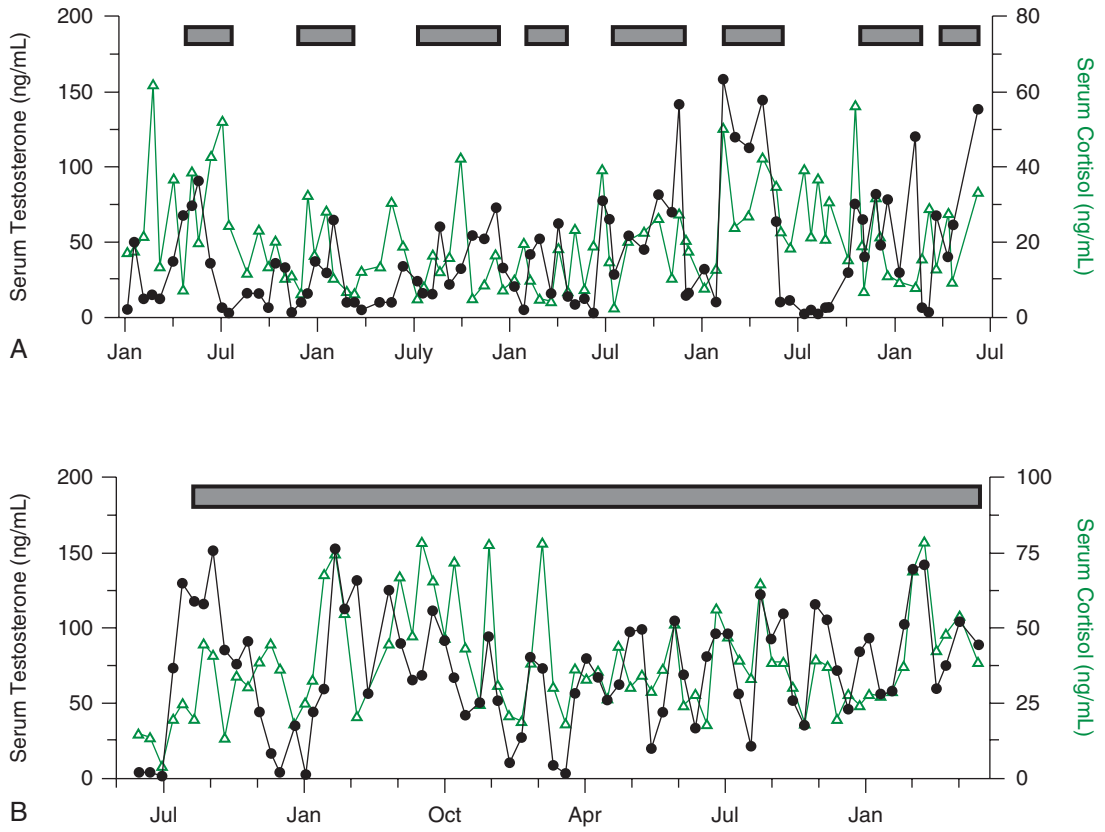
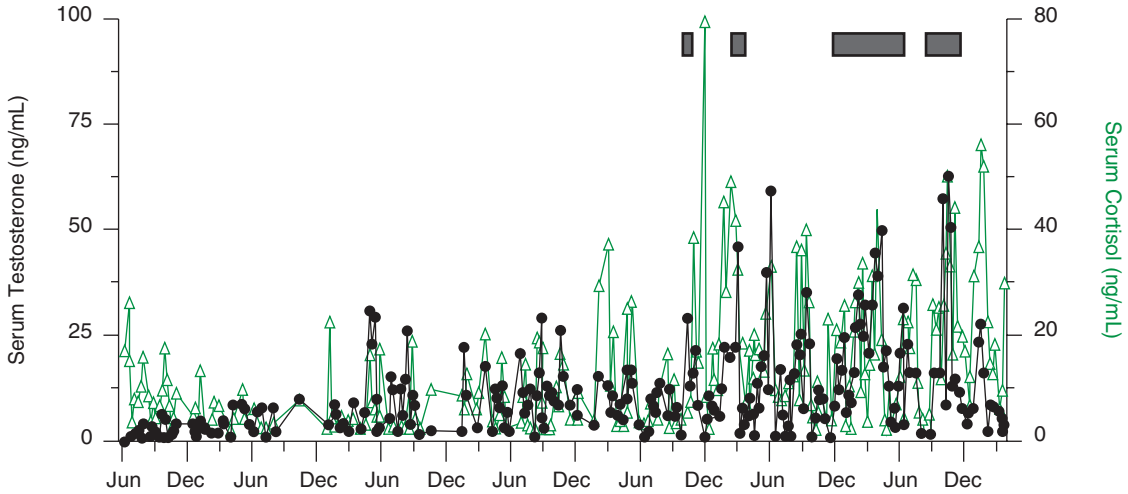


• **Fig. 42.13** Individual profiles of serum cortisol and testosterone in Asian elephant bulls exhibiting (A) multiple musth cycles each year and (B) nearly continuous musth symptoms. Musth periods are indicated by the black bars. (Adapted from Brown et al. 2007.)



• **Fig. 42.14** Individual serum cortisol and testosterone profile in an Asian elephant bull demonstrating initiation of musth; the first musth period was observed at 12 years of age, and multiple short musth periods were observed in the first year. Musth periods are indicated by the black bars. (Adapted from Brown et al. 2007.)

years of age. Early sexual maturation (i.e., increases in testosterone and musth activity) and multiple episodes of musth per year in some captive bulls may be as a result of overall better nutrition, lack of the presence of older, more sexually mature cohorts, and perhaps diminished environmental stressors (Brown et al. 2007).

Suppression of Musth

Due to the dangers associated with managing bulls in musth, especially in captivity, there is considerable interest in finding treatments that mitigate aggressive behaviours. Castration is not a practical option given the abdominal location of the testes, although it has

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been done. Musth is affected by nutritional status, so one strategy is to reduce water and food intake, although many bulls in musth reduce intake voluntarily. Musth bulls often are isolated, and in Asia they are kept on short chains away from other elephants and people. These practices raise ethical concerns, so alternative therapies that decrease androgen secretion and associated dangerous behaviours are being tested. GnRH agonists, such as leuprolide acetate, and antagonists (Detirelix) have been shown to reduce testosterone during musth but need regular injections (Brown et al. 1993, de Oliveira et al. 2004). Cyproterone acetate (an antiandrogen) has been given to Asian elephants in musth but with little effect on aggressive behaviours (Niemuller et al. 1998).

More recently GnRH vaccines have shown promise, including their use in elephant bulls, as a means of controlling reproductive and sex-related behaviours that are testosterone driven (De Nys et al. 2010, Somgird et al. 2016b). In Asian bulls, pituitary LH responses to exogenous GnRH are greatest in the premusth period (about 1–2 month before musth onset), which is when serum LH and testosterone are increasing (Somgird et al. 2016a). Therefore the assumption is that GnRH vaccination will be more effective when given before the predicted musth period to block the HPG axis; preliminary studies in Sri Lanka and Thailand have supported the theory. In Thailand, Asian bulls were given 600 μg of a GnRH vaccine (Improvac) approximately 2 months before expected musth, which resulted in a postponement of musth for 2 to 6 months, shortening or completely preventing the musth period (Somgird et al. 2016b). Serum LH and testosterone concentrations were decreased when anti-GnRH antibodies were elevated, and no UD was observed in musth bulls after completion of the vaccination programme (Somgird et al. 2016b). Similarly, African elephant bulls exhibited lower faecal androgen metabolite concentrations after GnRH vaccination, and musth behaviour was suppressed (Brown et al. 1993, Brown et al. 2007, De Nys et al. 2010). However, not all bulls respond to vaccination with a reduction in musth symptoms, which may be a learned behaviour. Long-term treatment with GnRH vaccines might also result in permanent immune castration (Lueders et al. 2014). A suppressive effect of a GnRH vaccine on reproductive organs was reported in a young Asian bull treated over several years, including reduced sperm production and size of reproductive organs (ampullae, seminal vesicles, testes, and penis; Lueders et al. 2017); thus it should be used cautiously.

Clinical Examination of the Reproductive System

The ability to conduct reproductive tract soundness examinations is important for the breeding management of captive elephants. Both male and female elephants should be trained to be subjected to transrectal palpation and ultrasonography, as well as physical restraint when needed. Elephants should not be allowed to swing their trunks or kick or walk away during the examination. Securing front and rear legs to a pole or other stationary object by ropes or chains or putting the elephant in an elephant restraint device may be needed to prevent injury to the elephant or examiner. If the elephant cannot be controlled, standing sedation with xylazine hydrochloride can be used.

Male reproductive organs, both testes and accessory sex glands, can be assessed by transrectal ultrasonography to determine the maturity of the male, as well as during the semen collection process (Hildebrandt 2000b, Imrat et al. 2014). Collected sperm should then be evaluated for quality – i.e., volume, colour, motility, viability – as a means to assess fertility (Thongtip et al. 2004b). But, as will be discussed later, it is important to note that a breeding bull who has produced a number of calves may not provide a good semen sample using artificial techniques (rectal message, electroejaculation, artificial vagina), whereas a bull who has never sired a calf may produce good quality samples. This is because the collection method itself may result in incomplete ejaculation or a poor mix of seminal fluids, which results in nonmotile spermatozoa.

In females, blood samples should be collected routinely (weekly or biweekly) for progestagen analyses (serum, faecal, urine, or saliva) to identify ovarian cyclicity status (Brown 2000, 2006, Hodges 1998, Lueders & Hildebrandt 2012). Transrectal ultrasonography is useful for evaluating urogenital tract health and identifying any reproductive pathologies, i.e., leiomyoma, fibroma, and cystic endometrial hyperplasia, which can cause reproductive failure (Hildebrandt et al. 2006b, Pringproa et al. 2015).

Reproductive Examination Tools

Ultrasonography

Ultrasonography is a technique used to evaluate the internal reproductive organ systems. Most ultrasonography used to monitor reproduction in male and female elephants is performed transrectally (85%) rather than transcutaneous (Hildebrandt et al. 2006b, Hildebrandt et al. 2006a) because the thickness of elephant skin (~3 cm) affects the sharpness of the ultrasound image. However, after 8 months of gestation, the fetus reaches a size that causes it to descend into the abdominal cavity, where it is no longer viewable. Thus in late pregnancy (after 16 months), transcutaneous ultrasound can be used diagnostically or to monitor fetal activity (Hildebrandt et al. 2006b).

Transrectal ultrasonography can be conducted when the elephant is standing or lying on its side without sedation. However, for the safety of the sonographer and the expensive equipment involved, the elephant must be well-trained or carefully tethered. The optimum ultrasound system in elephants is a portable, battery-powered, real-time B-mode, with the low frequency convex (3.5 MHz), middle frequency microconvex (5 MHz), and high frequency linear (7.5–10.0 MHz) transducers. Before performing transrectal ultrasonography, it is advisable to remove any faeces from the rectum and wash it out with warm water. To view organs, such as the urinary bladder, uterine body, vagina, vestibule, and seminal vesicles, a handheld ultrasound probe may be used, with the operator's arm extended to full length. However, images of deeper organs, such as kidney, testis, uterine horn, and ovary, require the use of an extension, as described by Lueders et al. (2017), to increase the length and depth of reach of the ultrasound probe.

Ultrasonography of the Male Elephant

Accessory sex glands and the intraabdominal testes can be imaged using transrectal ultrasonography. In adolescent males, this can be accomplished using a conventional ultrasound probe, whereas in adult bulls, a probe extension must be used because the testes are located approximately 0.9 to 1.5 m from the anus. Details of ultrasonography images have been described by Hildebrandt et al. (1998, 2000b). The ampullae, located bilaterally dorsal to the caudal part of urinary bladder, store spermatozoa produced by the testes. If the ampullae are full, the sonographic image will show a rounded echogenic structure filled with spermatozoa, which have a snowy appearance; this is common in the active testes of the bull. If the structure is anechoic, the ampullae will be filled with

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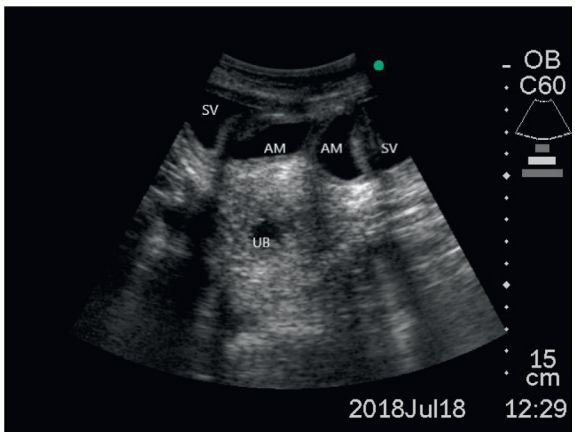

• **Fig. 42.15** Transrectal ultrasonography image of crosssectional plane of male reproductive organs. Ampullae are illustrated by two rounded hypoechogenic structures in the upper central region. Seminal vesicles are located lateral to the ampullae and filled with anechogenic seminal fluid. SV, seminal vesicle; AM, ampullae; UB: urinary bladder.

a spermfree secretion. Seminal vesicles are located lateral to the ampullae and filled with anechoic seminal fluid, which provides nutrients and a vehicle for sperm at ejaculation (Fig. 42.15). The testicular sonographic image is homogeneous and hypoechoic, with a hyperechoic border.

Ultrasonography of the Female Elephant

Monitoring of female reproductive organs is crucial for assessing ovarian activity in pregnancy diagnosis and to observe pathological changes in the reproductive tract that often occur in nulliparous older females (Agnew et al. 2004, Hermes et al. 2004). The vestibule is a tube-like structure that is present between the hindlegs and extends to the opening of the vagina. A crosssectional image of the vestibule shows a hypoechogenic mucosa surrounded by a hyperechogenic submucosa. The vestibular lumen is normally not detectable by ultrasonography. Longitudinal transrectal ultrasonography is very useful for vaginal diagnostic imaging. During the luteal phase, anechogenic mucus partially fills the vaginal lumen, whereas fragmented hyperechogenic mucus is observed in the homogeneous hypoechogenic vagina during oestrus. Oestrus is the most ultrasonographically active stage of the vagina and cervix, with significant changes in mucus echogenicity (Hermes et al. 2000).

A handheld ultrasound probe can reach the uterine body (5–10 cm) and externally fused uterine horn (or pseudouterine body, 50–70 cm), whereas the uterine horn can only be viewed by means of an ultrasound probe with an extension. Uterine lesions, such as benign tumours of the endometrium (leiomyoma) and cystic endometrial hyperplasia, can also be detected by transrectal ultrasonography. The ovary can only be reached by using the ultrasound probe extension; a high frequency transducer (5–7 MHz) is recommended to view the detailed structure of the ovary.

Endoscopy

Endoscopy is useful for reproductive tract health diagnosis and for conducting AI. A 3-m endoscope is guided through the genital tract via the vulva, vestibule, vagina, cervix, and uterus, where semen is deposited (Brown et al. 2004b, Thongtip et al. 2009). Endoscopy can also be used to visualise reproductive tract lesions, such as internal wounds or tumours. The endoscope can be easily damaged if the cow moves unexpectedly, however; so the technique should only be used in well-trained or sedated individuals.

Obstetrical Disorders

Retained Fetal Membranes

Normally, the fetal membranes are expelled within 10 to 12 hours after birth of the calf (Hildebrandt et al. 2006a). However, postpartum complications, including retained fetal membranes (RFM), can occur in elephants, resulting in metritis and reductions in milk production (Murray et al. 1996). Successful use of oxytocin, with or without oestradiol cypionate, and manual traction has been reported to successfully treat the problem (Murray et al. 1996, Brown et al. 2004b).

Dystocia

Dystocia is defined as a difficult birth, which in elephants can be caused by a number of conditions: excessive dam body weight and lack of exercise; advancing dam age; calf oversize; faulty disposition of the calf; small pelvis/birth canal; loss of flexibility in the pelvic region; muscle fatigue; hypocalcaemia; intact hymen obstructing the birth canal; oedema of the vestibule narrowing the birth canal; elephant endotheliotropic herpes virus infection; and cysts, polyps, and/or tumours of the urogenital tract (Hermes et al. 2008). In zoos, nulliparous females are generally considered to be beyond their normal useful reproductive life after approximately 30 years of age because of a greater risk of dystocia and stillbirth. Dystocia is suspected when there is: 1) no sign of impending parturition after progestagen concentrations have declined to baseline; 2) no relaxation of cervix based on transrectal ultrasonography; 3) no presentation of a fetus within 2 hours after rupture of the allantoic sac (Hermes et al. 2008, Hildebrandt et al. 2006a, Schaftenaar & Hildebrandt 2005) (Fig. 42.16). Of considerable concern is the tendency for older primigravida to experience dystocia and/or stillbirths. Over 50% of dystocias occur in primigravid cows older than 20 years of age or after prolonged barren periods (Hermes et al. 2008). As a management recommendation, nulliparous females in captivity are considered to be beyond their normal reproductive life after 25 to 35 years of age because of these increased risks (Hermes et al. 2004, Hildebrandt 2006).

When dystocia is diagnosed, veterinary intervention should be performed, such as transrectal manual massage of the vagina, cervix, and uterus to stimulate the Ferguson's reflex, which stimulates the release of oxytocin from the posterior hypophysis to stimulate myometrial contractions (Hermes et al. 2008, Schaftenaar 2013, Schaftenaar & Hildebrandt 2005). Medical management by administration of oxytocin and calcium can be used to stimulate uterine contractions; however, this should be avoided when there is obstruction of the birth canal. Oxytocin also should be used only in cows when it has been confirmed that the cervix is sufficiently relaxed and dilated and that the fetus is present in the birth canal in the correct disposition for birth (Hermes et al. 2008). There are several examples of how the administration of oxytocin resulted in uterine rupture because of insufficient cervical dilatation (Hermes et al. 2008). Published doses of oxytocin given IM or IV vary considerably, and range from 20 to 30 IU every 20 to 30 min, to 200 to 400 IU every 90 min. Based on one author's experience, a single dose of 40 IU oxytocin successfully induced parturition within about 20 minutes in an Asian elephant (Brown et al. 2004b).

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• **Fig. 42.16** A case of dystocia in an Asian elephant, indicating a large bulge in the perineal region with no delivery of the fetus 2 hours after rupture of the allantoic sac.

• **Fig. 42.17** Postparturient prolapse of reproductive tract organs (uterine, vaginal, and cervix) in an Asian elephant.

The only surgical option to correct a dystocia is vestibulotomy and, if necessary, a subsequent fetotomy (Schaftenaar 2013). Caesarean surgery is contraindicated in elephants because it can result in the rupture of the abdominal wound postoperatively from pressure and subsequent death of the cow from peritonitis (Hermes et al. 2008, Hildebrandt et al. 2006a, Schaftenaar & Hildebrandt 2005). Vaginal vestibulotomy is recommended to enable retraction of a dead fetus when it enters the cervical canal (Hermes et al. 2008, Hildebrandt et al. 2006a, Schaftenaar 2013, Thitaram et al. 2006). If the calf has progressed sufficiently into the vestibule, traction is usually sufficient to enable its delivery. Ventral oedema is often observed after a difficult birth and can be treated by encouraging exercise of the dam and the application of warm compresses (Murray et al. 1996, Brown unpublished).

Reproductive Tract Prolapse

Prolapse of the reproductive tract is rare, but when it occurs, it is a serious postpartum complication that often results from excessive physical activities after birth and is facilitated by an open cervix and uterine atony. Eversion and prolapse of the vagina, with or without the cervix and urinary bladder, will be apparent as a bulge in the perineal region. Rectal palpation and ultrasonography are helpful in identifying any organs entrapped within the urogenital canal. Cervicovaginal prolapse in an African elephant was first described by Mollel (1978). Successful correction of a cervicovaginal prolapse in a 47-year-old elephant that was not related to calving was described by Min Oo et al. (2016). Unsuccessful attempts to correct uterine prolapses are common, as in the reports of four cases in Thailand (Lorsanyaluck personal communication, Angkawanish et al. 2016) (Fig. 42.17), which showed varying degrees of cystocoele, uterine, vaginal, and cervical prolapse using ultrasonography. The protruded masses were repelled into the pelvic cavity after vestibulotomy, and a purse string suture used to close the end of vestibular tract to prevent prolapse reoccurrence.

Urogenital Tract Injuries

Injuries to the distal urogenital tract are possible after delivery of an oversized fetus, especially in primiparous mothers, and have been observed in both captive and wild elephants. Injuries are characterised by localised trauma and oedema of the distal urogenital tract after delivery of the calf. In captive elephants, the affected area can be observed endoscopically to assess the extent of damage, and if there is no serious damage to the vestibular canal, it can be flushed with sterile water and treated by the application of emollient creams to reduce the irritation and swelling. Tearing of the mucous membrane and/or underlying muscle layers may require parenteral and local antibiotic treatment. Fig. 42.18 shows a urogenital tract injury of a wild elephant in the Udawalawe National Park in Sri Lanka.

Female Infertility

Ovarian Acyclicity

Reproductive problems of captive elephants are well documented. In the African elephant in particular, a high rate of ovarian acyclicity, with persistent basal progestagen concentrations, hinders population sustainability (Brown 2014). In North America, 52%

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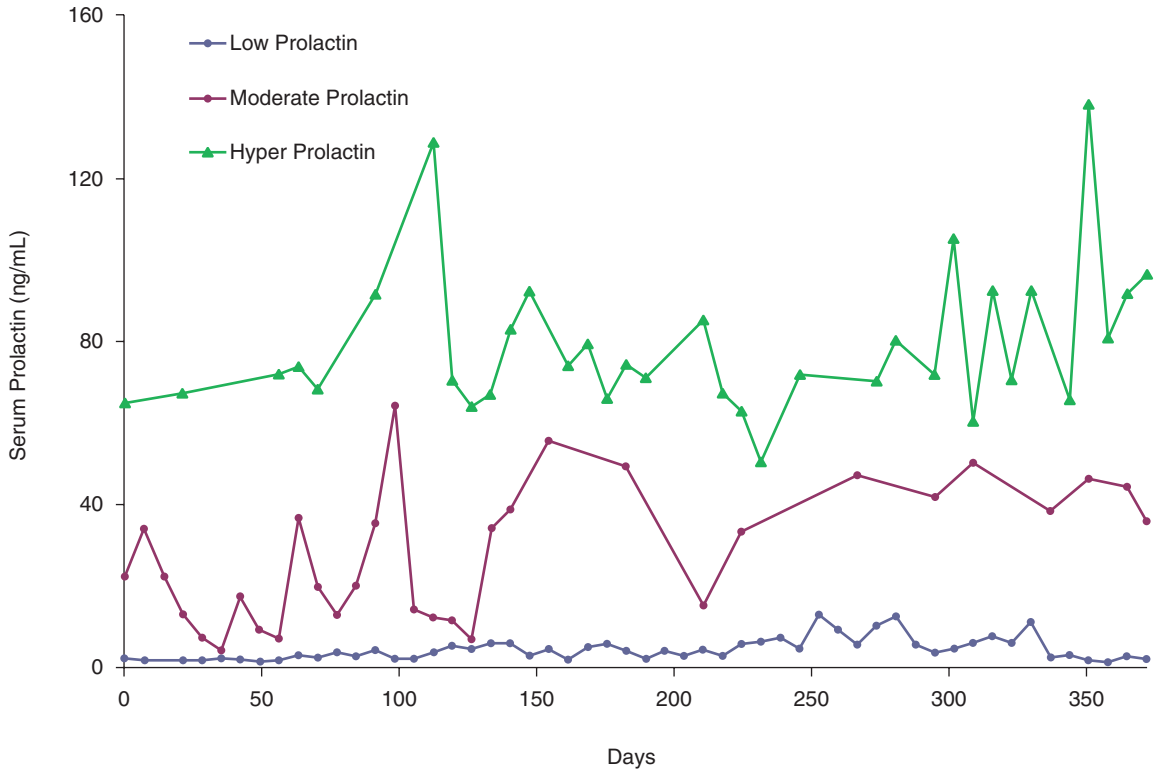
of African elephant females exhibit abnormal cycles, with 38% being completely acyclic (Brown et al. 2016). Comparatively, the acyclicity rate in Asian elephants is lower (< 20%) and affects mostly older cows (Dow et al. 2011a, Brown et al. 2016). Several conditions known to be associated with infertility in other species appear not to be related to abnormalities of the ovarian cycle in African elephants: for example, hyperandrogenism, hyperoestrogenism, hyper- or hypothyroidism, pituitary imbalances, and elevated cortisol concentrations (i.e., stress) (Brown 2014). The finding of no differences in anti-Müllerian hormone (AMH) concentrations between cyclic and acyclic elephants suggests that the problem is not due to a lack of functional follicles (Dow et al. 2011b). Notable problems associated with ovarian acyclicity in African cows are often related to abnormal prolactin secretion (Brown et al. 2004a, Yamamoto et al. 2010, Dow & Brown 2012, Prado-Oviedo et al. 2013). About half of acyclic African elephants in US zoos (the only population in which routine blood samples are collected for prolactin analysis) exhibit consistently low basal concentrations (Prado-Oviedo et al. 2013), whereas the other half produce levels that are five- to thirtyfold higher than in normal cyclic females (Fig. 42.19). This condition, called hyperprolactinaemia, is the most common disorder of the hypothalamic–pituitary axis related to infertility in women (Serri et al. 2003, Wang et al. 2012). As in other species, pituitary prolactin secretion in elephants is under inhibitory control by dopamine (Brown 2014). The dopamine agonist, cabergoline, is a common treatment for hyperprolactinaemia in women (Verhelst et al. 1999) and has been shown to effectively suppress prolactin secretion in hyperprolactinaemic elephant cows, but it has yet to result in the resumption of ovarian cyclicity in African cows even after a year of treatment (Morfeld et al. 2014a). No underlying physiological cause of hyperprolactinaemia has yet been found, but it does not appear to be related to abnormal production of cortisol, oestradiol, or thyroid hormones (TSH, T_3, T_4) (Brown et al. 2004a, 2014, Prado-Oviedo et al. 2013). Asian elephant cows rarely present with hyperprolactinaemia or prolactin-induced acyclicity (Brown et al. 2004a); this represents a significant species difference.

Several studies in the United States and Europe have applied epidemiological methods to identify factors in the zoo environment that are associated with reproductive disorders, and noted several differences between Asian and African elephants (Harris et al. 2008, Brown et al. 2016). For both species, the chance of a cow having normal cyclical ovarian activity decreases with age and is greater when they are housed in a mixed-sex herd. For African

• **Fig. 42.18** Urogenital tract injury subsequent to delivery in a wild elephant in Udawalawe National Park in Sri Lanka. (Photo courtesy of Dr. Vijitha Perera.)



• **Fig. 42.19** Individual profiles of abnormal serum prolactin concentrations in acyclic female African elephants demonstrating low (hypoprolactinaemia) and high (moderate and high hyperprolactinaemia) secretory patterns.

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elephants, social compatibility, herd stability, and enrichment are important for promoting normal pituitary–ovarian function, whereas social isolation has a negative effect (Brown 2016). Behaviourally dominant females that are identified as the herd 'peacekeepers' are more likely to be acyclic (Freeman et al. 2004). There also appears to be a social aspect to hyperprolactinaemia in African elephants, which is more prevalent in cows exposed to many social groups throughout the day, again possibly reflecting the negative effects of social instability (Brown et al. 2016). Last, the majority of Asian and African zoo elephants are overweight, based on their body condition score (Harris et al. 2008, Morfeld et al. 2014b), and in African cows, a high body mass index and body condition score have been related to ovarian acyclicity, altered metabolic function (insulin and the glucose to insulin ratio), and elevated leptin (Morfeld et al. 2014b). Interestingly, ovarian cyclicity in Asian cows is less affected by a high body condition, despite the fact that over three-quarters are considered overweight or obese.

Pathology of the Female Reproductive Tract

As reviewed by Hildebrandt (2006), older nulliparous cows or those that have not calved within approximately 10 years often develop urogenital tract pathologies. The most common are cysts and tumours, with some species differences. Endometrial hyperplasia and vestibular cysts are found in both species, whereas uterine leiomyomas are present only in the Asian elephants (Fig. 42.20), and vestibular polyps are observed only in African elephants. Vaginal cysts and neoplastic formations may be extensive, occluding the lumen, obstructing semen transport, and causing discomfort during oestrus and mating. These lesions may be a consequence of continuous ovarian cyclicity and hormone exposure in unbred females. In the wild, most elephant cows are either pregnant or lactating and thus experience comparatively few oestrous cycles in their lifetime. Consequently, rates of urogenital tract pathologies are higher in captive elephants (20%–80% in Asians and 10%–33% in Africans) and relatively rare in wild elephants (< 5%). As mentioned later in this chapter, GnRH vaccines, used to inhibit pituitary FSH and LH release, reduce ovarian steroidogenic activity and have been effective in resolving uterine cysts and tumours in older Asian elephants (Boedeker et al. 2012).

• **Fig. 42.20** Cystic endometritis hyperplasia with the fibroleiomyoma *(arrow)* in the uterine body and horns in nulliparous aged Asian elephant, which occluded the lumen, obstructed semen transport, and caused discomfort during oestrus and mating.

Male Infertility

Ultrasonographic examinations and semen evaluation of bull elephants has become a valuable tool for assessing sexual maturity (Hildebrandt et al. 1998, Hildebrandt et al. 2000b), detecting reproductive pathologies, and describing the ejaculatory process (Schmitt & Hildebrandt 1998). There appear to be fewer reproductive tract pathologies or asymmetrical reproductive aging in males than in female elephants, suggesting that captivity or being in prolonged nonbreeding situations does not have as significant an effect on bull reproductive health as it does on cows (Hildebrandt et al. 2000a). Although males exhibit fewer anatomically related lesions, they nevertheless exhibit a high rate of reproductive failure caused by a lack of libido or suboptimal sperm quality.

Lack of Libido

There is evidence that some causes of reproductive dysfunction can be related to the environment in which bulls are kept, grouping of bulls, and even the effect of overly dominant animal handlers. Housing multiple bulls together in a facility may reduce fertility in some subordinate bulls particularly in the human care setting, although limited studies have been done to support this as a universal response. One study found musth suppression was more common in groups of African than Asian bulls (Brown et al. 2007). During hot seasons, especially in Asian range countries, bulls are more likely to have poor libido and hence limited interest in breeding with cows.

Poor Semen Quality

All methods of semen collection in elephant bulls (e.g., rectal message, artificial vagina, electroejaculation) can result in samples of poor quality, usually in the form of low sperm motility or a high percentage of structural abnormalities (Fig. 42.21; Table 42.1). In extensive studies of captive Asian bulls, less than a third of ejaculates exhibited more than 60% motile spermatozoa, and many were contaminated with urine (Kiso et al. 2011, 2012, 2013), a common problem in elephant bulls resulting in damaged spermatozoa. Good motility ejaculates (> 65% motility) contained higher proportions of sperm with normal morphology, particularly intact acrosomes, compared with poor motility ejaculates. Furthermore, ejaculates with higher motility were of a larger volume with a lower sperm concentration. The influence of transrectal massage of the genital tract on seminal components has been reported (Imrat et al. 2013, Kiso et al. 2013). One- and two-dimensional gel electrophoresis revealed similar seminal plasma protein profiles between good and poor motility ejaculates, with the exception of a protein

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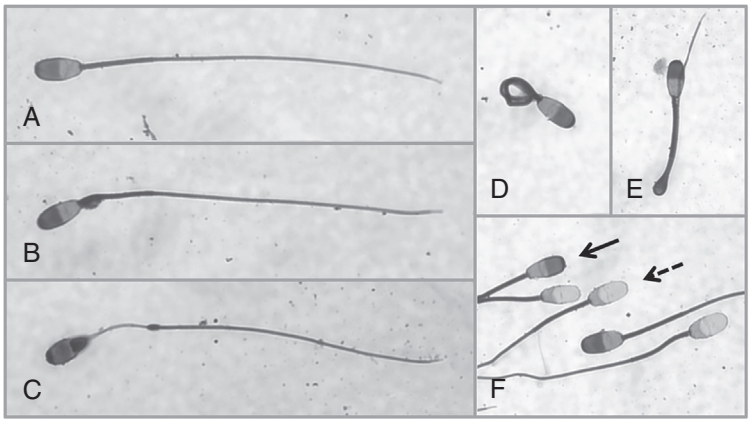

• **Fig. 42.21** Asian elephant sperm morphology and acrosomal integrity. (A) normal sperm; (B) proximal cytoplasmic droplet; (C) abnormal midpiece; (D) tightly coiled tail; (E) bent tail with cytoplasmic droplet; (F) acrosomal integrity (*solid arrow*: intact acrosome; *dotted arrow*: nonintact acrosome). Magnification 1000×. (From Kiso et al. 2013.)

TABLE 42.1 Mean (± SD) ejaculate characteristics of Asian and African elephant bulls

Semen Parameters	Asian Elephant (N = 75 Ejaculates)	African Elephant (N = 50 Ejaculates)
Total volume (mL)	48.3 ± 53.8	31.1 ± 38.7
Total motility (%)	25.8 ± 31.4	22.9 ± 30.3
Total progressive motility (%)	15.7 ± 26.3	18.5 ± 28.6
Normal morphology (%)	56.5 ± 26.9	45.6 ± 25.6
Normal acrosome (%)	17.3 ± 23.9	23.0 ± 31.0
Sperm concentration (X 10^9)	1.3 ± 0.8	2.3 ± 0.7
pH	7.2 ± 1.1	7.2 ± 0.9
Osmolality (mOsm)	256.2 ± 55.0	356.6 ± 124.4

From Kiso et al. 2011.

of approximately 80 kDa, that was abundant in 85% to 90% of ejaculates with good motility and absent in more than 90% of poor motility ejaculates. Mass spectrometry analysis identified the protein as lactotransferrin, which was confirmed by immunoblotting (Kiso et al. 2013). Lactoferrin is a fertility marker in human semen (Milardi et al. 2012).

Poor ejaculate quality can be attributed to infrequent ejaculation, causing the accumulation of senescent spermatozoa in the excretory ducts and ampullae. Increasing the frequency of semen collection has been shown to improve semen quality in some cases. However, semen characteristics also can be negatively affected by long-term management in captivity. Semen quality has been shown to be affected by age, season, and some nutritional and endocrine factors. Lower percentages of sperm with intact membranes and a higher frequency of detached sperm heads were observed with increasing age in nonmusth captive Asian elephants. It is important to note, however, that collection of poor quality ejaculates does not necessarily mean a bull is infertile and that many that produce very poor samples collected by artificial means successfully sire calves by natural mating.

Captive Breeding

Historically, *in situ* populations of elephants in Asia and Africa served as a source of new individuals for *ex situ* populations, a practice now recognised as irresponsible and unethical. Today, though not a solution, *ex situ* breeding of elephants can be viewed as a means of maintaining important populations as 'insurance' against environmental or anthropomorphic catastrophes. Globally, there are about 1000 African elephants in captivity, mostly in zoos, and upwards of 16,000 Asian elephants in zoos, circuses, sanctuaries, logging, and tourist camps, mostly in range countries. Few captive elephant populations are self-sustaining, due to high mortality and low birth rates, and may continue to be maintained through supplementation by wild capture and/or importation. This can put pressure on the already fragile wild populations, so most countries have banned the import and export of wild elephants. Increasing the reproductive output of captive elephants has been difficult, due to both physical and physiological challenges. Zoos in the United States typically average only 3.5 births to 5 deaths, for both species (Faust & Marti 2011a,b). Some problems are logistical, such as accessing fertile males and females together in the same facility. Others are physiological and related to ovarian cycle abnormalities, uterine pathologies, gestational difficulties, and bull subfertility. Finally, behavioural issues, such as mate incompatibility and poor libido, often hamper breeding success.

As reviewed by Thitaram (2012), breeding success in Asian range countries is also limited and can be influenced by work obligations and religious factors. In Northern and Southern India, elephants that belong to Hindu institutions often are prohibited from breeding. In other regions, captive Asian elephant cows historically bred with wild bulls, making those populations more sustainable. For example, Southern Indian forest camps that allowed elephants to forage at night to breed with either captive or wild bulls had a birth rate of 13% (Taylor & Poole 1998). In Myanmar timber elephants, mating of captive or wild bulls with captive cows resulted in an average birth rate of 7.1% per year per mature female during 1991 to 1995 (Lair 1997, Taylor & Poole 1998). In Sri Lanka, although not exposed to wild bulls, free contact between resident bulls and cows during grazing and bathing periods at the

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Pinnawala Elephant Orphanage has resulted in one of the most successful breeding programmes in Asia, with 3.9 calves born per year (Pushpakumara et al. 2016) and a birth rate of 20% annually (Taylor & Poole 1998). Therefore it appears that a major limiting factor in captive breeding is a lack of male–female contact in a favourable environment to support oestrus detection and mating. Across western and range country facilities, there are commonalities in what is required for successful breeding. These are:

- **Reproductively healthy bulls and cows.** Female elephants should be recruited for breeding at a young age (8–14 years) and kept reproductively active by breeding every 4 to 6 years to lower the risk of dystocia and stillbirth in later years (Brown 2014). Healthy parous females can reproduce into their 40s, and sometimes 50s. Multiple benign uterine tumours of the myometrium (leiomyoma) can develop if reproduction has not occurred within 10 to 15 years, especially in Asian cows (Hildebrandt et al. 2006a, Hildebrandt et al. 2006b); thus cows must be bred when they are young and frequently. The availability of experienced breeding bulls is crucial, and in many facilities, despite the presence of large numbers of adult bulls, only a few actually breed and sire calves (Thongtip et al. 2008).
- **Mate compatibility.** Placing males and females of reproductive age together is no guarantee of successful breeding. Some cows refuse the attention of some or all bulls, and vice versa. Males with a good temperament show better courtship behaviour and a willingness to mount and copulate. Fetomaternal blood incompatibility can cause haematological defects that can result in abortion and neonatal anaemia and may lead to calf loss (Bansiddhi et al. 2015). To reduce embryonic loss from fetomaternal blood incompatibility, testing of bulls and cows should be performed, consequently preventing the breeding of bulls and cows with high genetic relatedness (Thitaram 2009); unfortunately, in many breeding facilities, especially in range countries, genetic testing or the maintenance of reliable studbooks is not possible.
- **Correct timing of breeding.** Some bulls and cows can be kept together, thus ensuring breeding will take place at the correct stage of the oestrous cycle, which is a 2- to 5-day window at the end of the follicular phase and start of the luteal period (Vidya & Sukumar 2005). However, often males and females are kept separately to prevent injury to, or harassment of, cows by aggressive bulls. Oestrous detection to identify the optimum time for mating introductions can be performed by sequential progestagen monitoring (Brown 2006, Hildebrandt et al. 2011) or behaviourally (Thitaram et al. 2009). When daily blood sampling is possible, detection of the double LH surge is the most reliable means of estimating time of ovulation (Brown 2014).
- **Breeding locations.** Ideally, a quiet place away from the public should be used for breeding. In Asia, natural forested spaces are preferred, with animals mixed together after the working day has ended. However, it is not always possible to provide privacy in tourist camps or public zoos, so breeding often only occurs between elephants willing to breed in the presence of people.

Assisted Reproductive Techniques

Decades of advances in endocrine monitoring, ultrasonographic imaging, and semen collection techniques have led to a better understanding of the complex mechanisms involved in controlling reproductive function in elephants. Although adapting assisted reproductive techniques developed for domestic, laboratory, and other wildlife to elephants has not always been straightforward, a variety of methods now exist to aid breeding management, including semen cryopreservation and gonadal hormone control.

Semen Extension and Cryopreservation

Immediately after collection, semen can be extended to maintain viability. However, even excellent quality samples can deteriorate rapidly, and within 12 to 24 hrs the motility can be too low for use (Kiso et al. 2011, O'Brien et al. 2013). Typically, liquid semen storage at 35°C results in a sharp decline in spermatozoal quality after a few hours in both species, whereas spermatozoa held at 22°C and 4°C retained about half of their initial motility for up to 12 h (Kiso et al. 2011). The type of semen extender affects spermatozoal longevity. In Asian elephants, diluents providing a source of lipoprotein (i.e., skim milk or egg yolk) are the most effective (Kiso et al. 2012), with skim milk often resulting in better postthaw survival rates than egg yolk (Imrat et al. 2013). The inclusion of antioxidants to reduce DNA fragmentation (e.g., BullMax) also helps to preserve sperm longevity of Asian spermatozoa during storage (Imrat et al. 2012b). By contrast, African elephant spermatozoa generally retain viability longer than those of Asian elephants, and there is little difference in spermatozoal quality parameters using different extenders when extended elephant semen is stored at temperatures below body temperature (Kiso et al. 2011). For transport to recipient institutions, Asian elephant spermatozoa should be diluted in extenders containing egg yolk or skim milk, whereas spermatozoa from African elephants have no such requirement (Graham et al. 2004, Saragusty et al. 2005, Hermes et al. 2009, Saragusty et al. 2009, Kiso et al. 2011). The identification of considerable DNA damage and morphological degeneration in ejaculates after only 24 hrs of storage indicates that sperm ageing may be a primary contributor to inconsistent semen quality even at chilled temperatures (O'Brien et al. 2013). It has been suggested that Asian elephant spermatozoa are more susceptible to DNA damage those of other mammalian species (Imrat et al. 2012a) and that the amount of disulphide bonding and number of arginine–lysine residues in protamines likely influence the relative stability of sperm DNA (Gosalvez et al. 2011). Less crosslinking in elephant sperm may make them comparatively more fragile under *in vitro* conditions.

Cryopreservation of elephant spermatozoa was first attempted 30 to 40 years ago in African elephants (Jones 1973, Howard et al. 1986) and a little more than a decade ago in Asians (Hedrick & Schmitt 2001, Thongtip et al. 2004b). Initial protocols relied on freezing samples over liquid nitrogen vapour or forming pellets on dry ice; postthaw survival was generally poor (< 50%), more so for Asian than African elephants. As such, cell damage during the freeze–thaw process has been a major limitation to successful cryopreservation. During the process of chilling and freezing, the plasma membrane lipid bilayer of spermatozoa is altered in both composition and structure. The freezing of extracellular water results in hyperosmotic conditions that draw water out of the cell and can lead to altered membrane permeability, cell dehydration and shrinkage, and death. Several studies have attempted to enhance the postthaw survival of elephant spermatozoa by adding cryoprotectants (i.e., dimethyl sulfoxide, glycerol, ethylene glycol, and propylene glycol) before cooling and freezing (Kiso 2004, Thongtip et al. 2004b, Saragusty et al. 2009, Buranaamnuay et al. 2013), with glycerol generally being the most effective. In a preliminary study, fatty acid composition of Asian and African elephant spermatozoal

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plasma membrane was found to differ (Swain & Miller 2000). A lower proportion of polyunsaturated fatty acids in Asian elephant spermatozoal membranes suggested there might be less fluid. Increasing membrane fluidity by incubating with lipids, such as egg yolk or egg–phosphatidylcholine liposomes, helps elephant spermatozoa withstand such stresses (Saragusty et al. 2005). Today, most extenders for elephant semen cryopreservation contain a minimum of 15% to 20% egg yolk. In other species, treatment of spermatozoa with membrane stabilisers before cryopreservation has improved postthaw survival by modifying phase transition characteristics and increasing tolerance of the cells to freeze–thawing (Purdy & Graham 2004). Membranes with higher cholesterol or greater cholesterol:phospholipid molar ratios tend to be more tolerant to temperature changes during cryopreservation compared with membranes with lower cholesterol levels (Amann & Pickett 1987). In a recent study in Asian elephants, cholesterol loaded into spermatozoa by coincubation with cholesterol-loaded cyclodextrins (CLC; 1.5 mg of CLC/120 × 10^6 spermatozoa) increased membrane cholesterol concentrations and significantly improved postthaw spermatozoa motility and intact acrosomes – approximately 50% compared with approximately 25% with no CLC (Kiso et al. 2012). This percentage survival should be adequate for use with AI, as the three reported pregnancies occurred with postthaw sperm motilities of 45% to 60% (Thongtip et al. 2009, Hildebrandt et al. 2012a). A newer method of semen cryopreservation involves the use of directional freezing. In conventional freezing methods, ice forms at an uncontrolled rate and can damage cellular membranes. Using large volume (2.5 or 8 mL) cryogenic tubes (HollowTubes, IMT Ltd.), semen can be moved at a constant velocity through a linear temperature gradient, which results in better control of ice crystal formation and minimum damage to cells. Postthaw motility in excess of 50% has recently been obtained using directional freezing with semen extended in an egg yolk extender (e.g., Berliner Cryomedium: 2.41% (w/v) TES, 0.58% (w/v) Tris, 0.1% (w/v) fructose and 5.5% (w/v) lactose, 15.6% (v/v) egg yolk and 20 IU α-tocopherol/mL) with 7% glycerol (Saragusty et al. 2009, Hermes et al. 2013). A recent study has shown that faster freezer rates and higher thaw temperatures can significantly improve post thaw semen quality in Asian elephants (Arnold et al. 2017).

Traditionally, zoo elephant populations have been supplemented by the selective importation of females. This is in part due to the increase in husbandry requirements and costs associated with maintaining bulls, which limits the number of facilities willing to house them. As more zoos have become involved in breeding elephants by both AI and natural mating, the number of males in captive populations is increasing. With the advent of flow cytometric methods to separate sperm based on the DNA difference between X and Y chromosomes (Johnson et al. 1987), the technology now exists to preferentially select females. One obstacle to the practical application of this technique is identifying an extender that will optimise short-term storage of spermatozoa while simultaneously being compatible with flow cytometric sorting. Egg yolk added to semen extenders must be removed before sorting because it interferes with the uniform staining of DNA that is critical to separate X- and Y-chromosome cells (Johnson & Welch 1999). This additional processing and handling usually causes sperm damage and loss in viability. Nonegg yolk or reduced egg yolk–based extenders have shown some promise. Success has been achieved in sex-sorting of Asian elephant sperm utilising a MES-HEPES skim milk–based medium (Hermes et al. 2009). Another skim milk diluent, supplemented with only 4% egg yolk (INRA96), sustained sperm as well as media containing 20% egg yolk and thus can be used to extend ejaculates for shipment to centralised sperm sorting facilities (Kiso et al. 2011, Imrat et al. 2013). African elephant spermatozoa have no lipid requirement, so both TL-Hepes and Modena, which are devoid of egg yolk, should be effective in extending African elephant ejaculates for sorting (Kiso et al. 2011). Establishing a genome resource bank for elephants, if it includes sex-sorted samples from captive and wild bulls, could greatly enhance the genetic management of elephants under human care (Hermes et al. 2013).

Artificial Insemination

As the number of wild elephants continues to decline, maintaining healthy captive populations is vital. Because of difficulties in transporting a bull elephant from one institution to another for natural breeding, AI is an alternative tool for genetic management in this species. The technique was developed by a team from the Institute for Zoo Biology and Wildlife Research (IZW) in Germany, in collaboration with the Smithsonian Conservation and Research Centre in the United States (now the Smithsonian Conservation Biology Institute). The first calf conceived by AI was an Asian elephant at the Dickerson Park Zoo (US) in 1999, followed by two African calves at the Indianapolis Zoo (US) in 2000.

Successful AI revolves around several important factors. The cow must undergo a reproductive assessment to ensure that she is cycling normally (based on longitudinal progestagen analyses) and that there are no reproductive tract pathologies (based on transrectal ultrasound). The insemination must be timed correctly to coincide with ovulation. Weekly progesterone is monitored until the hormone levels decrease to baseline at the start of the follicular phase. Within about a week from the progestagen decline, daily blood sampling is initiated to identify an LH surge. Approximately 3 weeks later, inseminations should be performed to coincide with the day before and the day of the second ovLH surge (Brown et al. 2004b, Thongtip et al. 2009); a suitable fertile bull then needs to be identified as a semen donor. Breeding soundness examinations, using reproductive tract ultrasound examination, should be performed to evaluate the reproductive performance of the bull. After collection, semen should be diluted with an appropriate extender and kept chilled for transportation directly to the cow or cryopreserved so that it can be used later. Most births have resulted from the use of fresh or chilled semen, which limits AI effectiveness if good quality ejaculates cannot be collected on peak fertility days. Several calves have been produced successfully by AI using cryopreserved semen in African elephants, including with imported semen (Hildebrandt et al. 2012a). There have been several reported pregnancies from frozen-thawed semen in African elephants to date, including in a female inseminated using frozen semen from a wild African bull, resulting in a live birth in 2013 (Hildebrandt et al. 2012b and unpublished report). Another African cow conceived, but the pregnancy failed after 5 months (Dennis L. Schmitt, personal communication). The only success with frozen sperm in an Asian elephant was a pregnancy that ended in a stillborn calf after 17 months (Thongtip et al. 2009).

Two AI techniques for elephants have been reported: surgical via vestibulotomy (Schmitt et al. 2001) and endoscopic (Brown et al. 2004a, Hildebrandt et al. 2012b, Thongtip et al. 2009). Surgical AI is more difficult because of complications with wound infections, poor healing, and concerns for animal welfare, whereas endoscopic AI requires specialised accessory equipment, including a custom-made balloon catheter to distend the reproductive tract, and use of transrectal ultrasound to guide the endoscope/semen catheter. Both techniques require a tractable elephant, well-trained staff to control the elephant (with or without sedation), proper

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preparation of semen for insemination, and proper guidance of the catheter for insemination. Altogether, endoscopic AI is more convenient, less complicated, and presently is used as the standard protocol around the world.

The insemination is performed using an endoscope and ultrasonography to visualise the location of semen deposition. The nonsurgical procedure involves the use of a custom-made balloon catheter (2.5 cm diameter, 140 cm length) lubricated with nonspermicidal sterile gel, which is inserted into the vestibule to slightly distend the reproductive tract for optimal visualisation, followed by the passage of a flexible 3.0-m endoscope containing a customised disposable insemination catheter (3 mm diameter, 300 cm length) (Brown et al. 2004b). Both endoscopic and transrectal ultrasonographic visualisations are used to guide the insemination catheter towards the cranial vagina, just caudal to the cervical os or intracervically, where semen deposition can be visualised ultrasonographically. The sterile 1.7- or 3-meter endoscope with semen catheter is inserted through the vaginal os into the vagina. An AI catheter is introduced through the endoscope and extended semen deposited in the reproductive tract, ideally in the cervix or uterine body. However, if the cervix is not dilated, or it is impossible to penetrate the cervix, vaginal deposition may be effective. One of the main obstacles in nulliparous elephants is inserting the catheter through the vaginal opening and avoiding the adjacent two blind pouches (Hildebrandt et al. 2006b).

As a management tool, AI has been in use for nearly 20 years; however, the utilisation of this technique has been hampered by: 1) the inability to consistently obtain good quality ejaculates; 2) poor spermatozoal survival after liquid storage and transport; and 3) poor postthaw recovery of cryopreserved sperm. There is little doubt that use of AI is a significant management tool for the breeding of captive elephants, but maximal effect will not be attained until good quality semen samples can be reliably obtained or cryopreserved for use with AI. It is not as simple a procedure as in most domestic livestock, and it requires an extensive amount of planning, coordination, hormonal monitoring, training, financial resources, and staffing, as well as collaboration with multiple bull holding facilities (Brown et al. 2004a). It is important to emphasise that the pregnancy rates are not as high as with natural mating (< 20%).

Contraception and Reproductive Control

Ovarian and testicular functions are under hormonal control, and thus, methods that alter hypothalamic-pituitary-gonadal (HPG) activity can be used to manipulate reproduction. Under certain managed situations, there is sometimes the need for a temporary or even permanent cessation of breeding to prevent overpopulation in restricted areas without disrupting herd dynamics (Delsink et al. 2002). It also prevents gonadal steroid-induced reproductive disorders, such as reproductive tract cysts and tumours, that can result from continuous ovarian cyclicity in older, nulliparous females (Boedeker et al. 2012). For example, in well-managed, private South African nature reserves and national parks, elephant populations increase about 3% to 6% annually. Hence, there is an urgent need for efficient population management protocols to keep the number of elephants at an ecologically acceptable level. Hormonal agonists and antagonists that disrupt the HPG axis have been used for reproductive control in elephants. Oestrogen implants have been shown to prevent pregnancy in wild African cows. However, prolonged periods of induced 'oestrus' resulted in herd disruptions, with treated females being harassed by bulls. Moreover, some young calves were harmed when cows, which were harassed by bulls, stopped lactating. Therefore to suppress the HPG axis and control sex hormone production, immunocontraceptive methods have been developed that are safe, effective, and do not disrupt the social structure of the herd.

Immunocontraception

Porcine Zona Pellucida Vaccine

The most widely applied contraceptive agent in elephants is an immunogenic preparation of porcine zona pellucida (pZP) harvested from pig ovaries (Delsink et al. 2002, 2006, Fayrer-Hosken et al. 1999). In one field study in Africa, cows were vaccinated with an initial dose of 400 µg IM, followed by two 200 µg boosters at 5 to 7 week intervals, and then subsequent 200 µg annual boosters (Ahlers et al. 2012). This vaccination programme decreased the population growth rate from 10% to 0% in just a few years. PZP vaccine has many advantages, including: 1) a high contraceptive efficacy (at least 90%) with no negative effects on social behaviour; 2) safe and with no adverse effect on health or pregnancy; 3) highly cost effective; and 4) not entering the food chain (Delsink et al. 2007, Delsink et al. 2013, Kirkpatrick et al. 1996). However, pZP vaccination only blocks the sperm receptor on eggs and does not appear to have an effect on suppressing ovarian cyclicity.

Gonadotropin-Releasing Hormone Vaccine

Gonadotropin-releasing hormone (GnRH) vaccines stimulate the production of anti-GnRH antibodies that block the binding of endogenous GnRH to gonadotrophin receptors in the pituitary gland (Conforti et al. 2008) and inhibit the release of FSH and LH from the anterior pituitary, thereby causing the cessation of cyclic ovarian steroidogenic activity. They have been used in domestic and wild species for contraceptive and aggression-related behavioural control (De Nys et al. 2010, Somgird et al. 2016a).

The most useful application of GnRH vaccination in elephants has been related to the treatment of reproductive tract pathologies, which develop in older females that exhibit continuous ovarian cyclicity. In the first study, a CpG motif–based adjuvant in a recombinant GnRH vaccine (Repro-BLOC, Amplicon Vaccine, LLC, Pullman, WA) suppressed ovarian cyclical activity and resolved haemorrhage and anaemia associated with a vascular reproductive tract tumour in a 59-year-old Asian elephant (Boedeker et al. 2012). Synthetic oligodeoxynucleotides (ODNs), containing unmethylated CpG motifs, directly stimulate B cells and plasmacytoid dendritic cells, thereby promoting the production of cytokines and the maturation/activation of antigen-presenting cells (Klinman 2006). These activities enable CpG ODNs to act as immune adjuvants, accelerating and boosting antigen-specific immune responses by 5- to 500-fold over traditional Freund's adjuvants. Ten years after initial vaccination, the cow continues to lack distinct ovarian cycles but is otherwise healthy. In follow-up studies, administration of three doses of GnRH vaccine (800 µg, Bopriva, Pfizer Animal Health, Australia) 4 weeks apart induced anoestrus in cyclical captive Asian cows based on progestagen analyses (Rajapaksha, Pushpakumara, Perera & Brown, unpublished data). Continued trials in the United States utilise commercial GnRH vaccines, Bopriva and Improvest (Pfizer Animal Health), at doses of 400 to 1000 mg, boosted at monthly intervals for 3 to 5 months. Effects are fairly short term, with most cows resuming cyclical activity within 1 to 6 months of the last booster (Boedeker and Brown, unpublished data). A recent GnRH vaccination (1000 µg, Imrovac, Pfizer Animal Health) study conducted in wild and captive African bulls has shown that regular vaccine boosters can be used as contraceptive method in this species (Lueders et al. 2017).

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PART 7

Assisted Reproduction

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Artificial Insemination

TIMOTHY J. PARKINSON AND JANE M. MORRELL

The successful use of artificial insemination (AI) as a means of animal breeding relies upon three major premises: first, that spermatozoa can survive outside the body; second, that they can be reintroduced into the female genital tract in a way that results in an acceptable conception rate; and third, that the fertile period of the female can be identified.

The degree to which these underlying premises can be fulfilled dictates the success with which AI can be applied to an animal species. For example, in cattle, the spermatozoa can be preserved outside the body almost indefinitely (after cryopreservation). A technically straightforward intrauterine insemination means that the number of spermatozoa needed for each insemination dose is low; hence, each ejaculate can be used for breeding many females. Conception rates with AI are identical to those of natural service, whilst the visible oestrous behaviour of cows means that detection of the fertile period is not difficult. Hence, in this species, in which all three premises are fulfilled, the use of AI is widespread. Conversely, in many other species, in which one or more of the premises are less adequately fulfilled, AI is less successful and therefore less widely used.

AI regimens have been developed for most domestic and many semidomestic species. It is routinely practiced in cattle, sheep, pigs, goats, fowl, turkeys, salmon, and trout and is used in dogs, domestic foxes, buffalo, horses, and even bees. Of these, cattle, pigs, and sheep/goats account for the vast majority of mammalian inseminations. The use of AI is essential in turkey breeding as natural mating is not possible in this species, so that very large numbers of inseminations are performed. AI in salmonid farming is also very widespread. The discussion of AI in this chapter will be limited to the major domestic mammals.

Advantages and Disadvantages of Artificial Insemination

Artificial insemination offers several potential advantages over natural service. Historically, the most important reason was the control of venereal diseases, particularly in cattle. Nowadays, the most commonly advocated reason is as a means of genetic improvement.

The potential for AI to be used to control venereal disease was a major impetus to the development of cattle AI in the UK during the 1940s. At that time, the venereal pathogens *Tritrichomonas foetus* and *Campylobacter fetus* subsp. *venerealis* were epizootic in most cattle populations, but in the UK, in common with most countries in which bovine AI was introduced in the face of trichomonosis and campylobacteriosis, these pathogens were virtually eliminated by its use (see Chapter 24). However, the converse is also true: uncontrolled use of sires in AI can disseminate disease. Many diseases are transmissible through semen, including not only the classic venereal diseases, but also other conditions that would not generally be regarded as primarily venereal (Roberts 1986). Rigorous monitoring of the health of AI donor sires is therefore regarded in many countries as an integral part of national disease control programmes.

The use of AI as a means of genetic improvement stems from the fact that, in most food-producing animals, each ejaculate can be divided into many insemination doses, such that each sire can potentially be used to breed a very large number of females. Hence, the total number of sires needed is reduced, with a consequential increase in the selection intensity that can be applied to the male side. In dairy cattle, only the best 1% of cows is selected as potential bull mothers and only about the best 1% to 3% of their male progeny eventually become sires of the next generation. In beef cattle and pigs, the selection intensity is not quite so great but, nevertheless, is very much more intense than can be achieved in natural breeding.

Direct genetic selection of sires is only one of the ways in which AI can be used for achieving genetic improvement, inasmuch as AI allows rapid dissemination and/or substitution of new breeds. In the UK, AI was one of the main means whereby the Friesian breed of cattle displaced the indigenous British dairy breeds. Subsequently, AI has also been the means by which the Friesian has been displaced by the Holstein. In such breed substitution programmes, AI can be used to change the gene pool of a national herd rapidly; a technique that is also used for upgrading unimproved cattle in remote areas. In this process, AI has the advantage of being both cheap and simple, inasmuch as local distribution of extended and cooled semen from a small number of imported sires is within the economic capabilities of even the poorest countries.

International trade in livestock is also facilitated by AI. Improved stock can be imported in the form of semen for AI rather than having to move animals themselves. By this means, many of the problems of acclimatisation, such as a lack of resistance to local diseases, can be eliminated. Importing semen also allows the importing country to exert a far greater level of effective control over the health status of the donor sires than if the livestock itself were imported.

The third major advantage of AI is the reduction in the number of sires that individual farmers need to maintain. The males of agricultural species generally require accommodation in which they can be segregated from the breeding females, so that breeding can be controlled; these buildings should also preclude, as far as

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possible, injury to farm staff. The significant housing and labour costs involved in keeping such animals can be obviated by the use of AI. Moreover, farmers generally have access to genetic material through AI centres that would be far beyond their financial ability to buy outright.

Nevertheless, although AI carries many advantages over natural breeding, the technique is not without drawbacks. Detection of the fertile period in the female oestrous cycle is potentially the most problematic aspect of AI programmes. In cattle, the prominent behaviour of oestrous females in which they repeatedly mount each other allows relatively accurate human identification of the fertile period. Likewise in pigs, receptive females 'freeze' when pressure is applied to their back. However, in most other species, detection of the most fertile period is less easy. In such species, detection of oestrus therefore requires the presence of infertile (e.g., vasectomised) males, or the timing of oestrus must be controlled by pharmacological regimens (e.g., oestrus synchronisation/induction; see Chapter 8), or management procedures (e.g., timing of weaning in sows). Thus for ewes, which do not normally display any signs of oestrus in the absence of a male, AI requires either the presence of vasectomised rams to detect oestrus or pharmacological manipulation of oestrus to define the timing of the fertile period. Hence, detection of the fertile period of the ewe is, to a greater or lesser extent, a costly procedure, thereby detracting from the appeal of AI in this species. It may therefore be considered that an economic 'trade-off' exists in such species between the genetic advantages conferred by the use of superior AI sires on one hand and the costs of maintaining teaser males or pharmacological manipulation on the other.

Once oestrus has been identified, the female animal has to be restrained for insemination, which generally requires separation from the herd or flock and holding in specialised pens. The process of insemination also requires trained personnel with a certain degree of technical proficiency, as with insemination of sows; however, in some cases the procedure may be technically demanding, as in the case of laparoscopic intrauterine insemination of ewes.

It is also necessary to log insemination dates into an adequate recording system so that expected dates of return to oestrus are known and in order to allow birth dates to be calculated, thus allowing appropriate observations to be made. In addition, the identity of the sire needs to be recorded (and his pedigree known) to avoid inbreeding. Some form of positive pregnancy diagnosis is advantageous, especially when males are not present in the herd, to ensure that non-returns to oestrus signify pregnancy rather than anoestrus.

The value of AI as a rapid means of transmission of the genes of superior sires has already been identified. However, a corresponding disadvantage exists, inasmuch as genetic faults can also be widely disseminated if they are present in an AI sire. Dominant traits should rarely be transmitted in this way, but recessive traits may be very widely transmitted, especially if the recessive gene is present in the general population at such a low incidence that many individuals may have to be bred before the condition is expressed in a homozygous progeny. Hence, AI programmes should be underpinned by an efficient reporting system for monitoring abnormalities in the progeny, with clearly defined criteria for the withdrawal from use of sires that carry deleterious genes. For example, in cattle, achondroplasia is transmitted as a simple recessive gene (Jones et al. 1978) that, when present in the homozygous condition, causes failure of long bone development, resulting in the birth of so-called 'bulldog' (achondroplastic) calves (see Chapter 9). The incidence of this gene in the general cattle population is so low that the birth of one or two calves with this deformity is regarded as sufficient reason to slaughter the bull and withdraw all stocks of its semen. Spastic paresis, which is probably genetically transmitted, is dealt with in a similar manner (Keith 1981). More recently, a complex vertebral malformation was identified in Holstein cattle in many countries (Agerholm et al. 2001), but affected sires could be effectively removed from the breeding pool by rapid identification through breeding records and genomic analysis (Thomsen et al. 2006). Moreover, it is also possible to disseminate conformational faults which, although not initially undesirable, can still have a significant effect upon the progeny (e.g., poor hind leg/foot conformation or poor udder attachment in cattle).

Concern over the possibility of inbreeding and reduction of the effective population of a breed has been suggested as a consequence of the widespread use of AI from a small number of highly selected blood lines. There is evidence that this may be taking place, particularly within the Holstein breed of cattle. A number of studies have identified real or potential negative effects of inbreeding on traits such as dystocia and reproduction (Adamec et al. 2006, McParland et al. 2007a), culling rate (Sewalem et al. 2006), and production traits (Biffani et al. 2002, Croquet et al. 2006), but generally not on type/conformational traits. Estimates of coefficients of inbreeding in Holstein cattle have shown a period of rapid increase during the 1990s, whilst the numbers of effective contributors to the breed is declining (Sorensen et al. 2005, Koenig & Simianer 2006, Kim & Kirkpatrick 2009). The rate of increase of inbreeding may have reduced in recent years (Stachowicz et al. 2011). This trend is also seen in beef cattle such as the Hereford and Charolais breeds (McParland et al. 2007b). McParland et al. (2007a) noted that although these effects were statistically significant, they were small and were unlikely to cause great financial loss at present levels. However, others advocate a more active role in the management of inbreeding (Kearney et al. 2004, Colleau & Moureaux 2006, Haile-Mariam et al. 2007).

Preparation of Semen for Use in Artificial Insemination

The methods for collection of semen from domestic mammals are described in Chapter 35. In most AI regimens, semen evaluation is limited to measuring numbers of spermatozoa, motility, and, usually, morphology. More sophisticated analyses may be used in determining whether an individual sire produces semen of a sufficiently high quality for acceptance into an AI programme, but such evaluations are rarely carried out on day-to-day collections of semen. Unless the semen is to be directly inseminated without delay into a single female, it is then extended and either cooled or frozen. Direct inseminations are performed most commonly in the bitch, usually in response to some incapacity of the sire that precludes normal mating (Roberts 1986) or the mare with chronic endometritis (Asbury 1986). Even in these circumstances, it is more common to place the semen in an extender before insemination. The major properties of semen extenders (Watson 1979) are described here.

Extension

The ejaculates of most domestic animals contain more spermatozoa than are needed for achieving a pregnancy. Hence, by diluting the semen, it can potentially be used for several inseminations. In species such as the dog and the horse, the whole sperm-rich

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fraction of the ejaculate is diluted and cooled, then used either for sequential inseminations of the same female over her extended oestrus period or after various determinations of the fertile period (Jeffcoate & Lindsay 1989, Brinsko & Varner 1993). In food animal species, the ejaculate is generally diluted so that it can be used to inseminate many females.

In either case, the maximum degree of dilution is determined from the minimum number of spermatozoa and the volume of inseminate that is deemed necessary to achieve acceptable pregnancy rates. These factors are themselves determined by the site of insemination, the survival of spermatozoa in extender, and the idiosyncrasies of individual species and sires. In general, when an intrauterine insemination can be achieved, the minimum numbers of spermatozoa are one or two orders of magnitude lower than for an intracervical insemination, which is itself one or two orders of magnitude lower than for an intravaginal insemination. Hence, when widespread use of sires is required, a great advantage exists in devising methods of achieving intrauterine insemination, even when this requires as complex a procedure as laparoscopic insemination, as in the ewe.

Addition of Volume

Insemination doses must be prepared in a volume that is a compromise between ease of handling and an appropriate volume for the site of insemination. Thus for ovine intracervical inseminations, minimising volume is important to reduce retrograde loss from the cervix (Evans & Maxwell 1987), whereas for porcine intrauterine inseminations, a minimum volume of 50 mL is required to spread the semen through the capacious uterus of the sow (Reed 1982).

Dilution of semen is not entirely straightforward, for mammalian spermatozoa placed in simple diluents exhibit an initial increase in motility, which is then rapidly followed by a loss of motility and increase in vital staining (Mann 1964). This phenomenon, known as the 'dilution effect', represents a loss of cell viability, probably through leaching of structural components of the cell membrane. Although it was of great concern among the early practitioners of AI, the use of extenders containing macromolecules such as proteins or polyvinyl alcohol was subsequently found to abrogate the dilution effect (Suter et al. 1979, Clay et al. 1984).

Buffering

Spermatozoa have a narrow range of tolerance to changes in pH, so provision of buffering capacity is necessary. Buffering is especially important when the semen is only to be cooled and not cryopreserved, as the metabolic activity of cooled spermatozoa remains appreciable (Salisbury et al. 1978). Although in many diluents the major volume component is also the major buffering agent, buffers are a minor constituent of some diluents. Simple buffers are effective, with citrate originally being widely used (Willett & Salisbury 1942). Phosphate-buffered saline is rather less suitable, as it predisposes to head-to-head agglutination of sperm. More recently, organic buffers have been used. Tris (tris(hydroxymethyl) aminomethane) is probably the most widely employed of such buffers, but the successful use of many similar materials (e.g., TES, HEPES, Tricene) has been described. The proteins contained in skimmed milk products also provide considerable buffering capacity to diluents. More recently, there has been a move away from using material of animal origin in semen extenders because of the risks of inadvertently transmitting disease. Lecithins from plants and liposomes have been used as substitutes for skimmed milk and egg yolk in semen extenders.

Maintenance of Osmotic Pressure

Seminal plasma has an osmotic pressure of 285 mOsmol, although spermatozoa can tolerate a moderate range of tonicity (Foote 1969). Some debate has centred on whether spermatozoa respond better to a slightly hyperosmotic (Foote 1970) or isosmotic diluent, with the former being generally favoured. Apart from the osmotic activity of the ionic component of diluents, a substantial contribution is made by proteins and, particularly, by sugars, which are added to provide nutrition for the spermatozoa or to contribute to the cryoprotective properties (Watson 1990) of the diluent.

Provision of an Energy Substrate

Most diluents make some provision of energy substrates for sperm. In general, simple sugars such as glucose, fructose, mannose, and arabinose are suitable substrates, although the rate at which these sugars are metabolised varies substantially between species (reviewed by Bedford & Hoskins 1990). Lactose, which is present in milk-based diluents, is not metabolised to any appreciable extent. However, egg yolk, also a component of many diluents, provides many substrates for the metabolism of spermatozoa (Salisbury et al. 1978). The provision of energy is relatively less important when semen is to be frozen, for the spermatozoa will only remain active for a few hours at most before freezing suspends metabolic activity. However, when the metabolism of spermatozoa has to be sustained for several days (e.g., if cooled semen is to be used) provision of energy is important.

Antimicrobial Activity

Antibiotics are added to most semen diluents as a prophylactic measure against the transmission of pathogenical bacteria and to reduce the load of nonpathogenical organisms that contaminate the semen. In cattle AI, benzylpenicillin and streptomycin (Melrose 1962) are the most widely used antibiotics, for these are efficacious against *C. fetus.* Most other antibiotics either fail to control this organism or are directly detrimental to sperm. Concern over the potential transmission of *Mycoplasma* and *Ureaplasma* species in bovine semen has led to the incorporation of lincomycin and spectinomycin (Almquist & Zaugg 1974) into semen diluents in an effort to control these organisms. There is evidence that the efficiency of antibiotics may be reduced in the presence of some components of diluents, notably egg yolk (Morgan et al. 1959); hence, the practice in some bovine AI centres is to preincubate the raw semen with antibiotic cocktails before the main dilution occurs. This procedure is commonplace in the United States but is rarely undertaken in Europe. Nowadays, there is increasing concern over the spread of antimicrobial resistance, which raises questions about whether the inclusion of antibiotics in semen extenders is justifiable because it has a prophylactic rather than a therapeutic purpose. Alternatives to antibiotics, such as the removal of contaminating bacteria during processing of the semen, would be preferable (Morrell & Wallgren 2014).

Extending Life Span

The life span of spermatozoa of most other species can be prolonged more conveniently by:

- Cooling to a temperature well below ambient;
- Freezing (cryopreservation); and
- Suspending the metabolic activity of the spermatozoa while maintaining it at ambient or cooled temperatures.

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Cooling and Cold Shock

Semen can be stored in a liquid form if the metabolic activity of the spermatozoa is reduced by cooling. The process of cooling from body temperature to 5°C results in considerable damage to the cells unless they are protected from the effects of the 'cold shock'. The development of cold shock is exacerbated by rapid cooling rates but cannot be entirely prevented even by slow cooling.

Cold shock results in damage to cell membranes, causing leakage of intracellular potassium, enzymes, lipids, cholesterol, lipoprotein, and adenosine triphosphate (ATP) (Salisbury et al. 1978). How this affects spermatozoon function remains incompletely understood, although it is clear that most of the effects are mediated through changes in the properties of cell membranes. Lowering temperature causes membrane phospholipids to change from a fluid to a gel phase, which, because this occurs at different temperatures for different structural lipids, can lead to phase separation. As a consequence, membrane proteins become irreversibly clustered, leading to loss of function (De Leeuw et al. 1990). Thus cold-shocked spermatozoa are more permeable, especially to calcium (Robertson & Watson 1986). The membranes are also more fusogenic, leading to alterations in the capacitation process (Johnson et al. 2000). Together, these changes are referred to as 'cryocapacitation'. Species differences and probably differences between individual sires depend on the composition of the membranes: ratios of different phospholipids and the concentration of cholesterol in the membranes appear to be critical determinants of the response to cold shock.

The most effective way of protecting spermatozoa against the detrimental effects of cooling is by the inclusion of egg yolk or milk in the diluent. Diluents for cooled storage of semen therefore typically contain approximately 20% of egg yolk plus a buffer. Skimmed milk, whole milk, and coconut milk have also been used successfully, although egg yolk is by far the most commonly used additive (Vishwanath & Shannon 2000). Whole milk contains a protein, lactenin, which is spermicidal, so milk for use as a semen diluent must be heat-treated (e.g., in the skimming process) to inactivate this toxic factor (Flipsse et al. 1954). Chemically defined extenders containing phosphocaseinate have now been developed (Batellier et al. 1998) and are tending to replace skimmed milk extenders. Early diluents were based on phosphate buffer, but citrate soon proved to be better, probably because it improves the solubility of egg yolk proteins. More recently, zwitterion buffers have also been used; Tris has been the most widely used but good results have also been achieved with TES (N-tris (hydroxymethyl) methyl-2-aminoethane sulphonic acid) or TEST (Tris titrated with TES) (Holt 2000).

Detailed understanding of the mechanisms by which egg yolk and other natural products prevent cold shock have been elusive, with the result that it has been difficult to produce chemically defined diluents. Early work identified that the key components of these materials were lecithins, proteins, lipoproteins, and similar complexes of large molecules (reviewed by Salisbury et al. 1978). Studies in the 1970s and 1980s (e.g., Watson 1976, 1981, Foulkes 1977) showed that the low density lipoprotein (LDL) fraction is the key component of egg yolk, with lipoproteins probably being the most important (Watson 1990). In the last 20 years it has been shown that lecithins from plants, e.g., soy lecithin, also have a protective effect and can be substituted for egg yolk (Hinsch et al. 1997) at least for some species. The evolution of soy-based extenders has been reviewed recently (Layek et al. 2016).

It has been suggested that LDL stabilises the spermatozoal membrane (Watson 1975), or protects the spermatozoon by forming a protective layer over its surface (Quinn et al. 1980) or by replacing membrane phospholipids that become lost or damaged (Foulkes et al. 1980). LDL, glycolipids, and cholesterol become incorporated into membranes, reducing their tendency to change from a liquid to a gel phase during cooling (Isachenko et al. 2004). Further studies focused upon the interaction of LDL with protein components in the seminal plasma (Vishwanath et al. 1992). Bergeron and Manjunath (2006) developed this idea further based upon their work on bovine seminal plasma proteins (BSP). They suggested that, *in vivo*, BSP bind to the membrane of the spermatozoa during ejaculation, facilitating the process of capacitation in the female tract. During storage, BSP binds to spermatozoal membranes and causes loss of phospholipid and cholesterol, thereby inducing one of the main mechanisms of cold shock. It is suggested that LDL, particularly phosphatidylcholine, binds BSP in the extender, preventing its interaction with the spermatozoa and hence protecting the membranes from damage.

The possibility of including liposomes in extenders to stabilise spermatozoal membranes during cryopreservation is also attracting attention (Röpke et al. 2011). Phosphatidylcholine liposomes have been incorporated into commercial extenders and used successfully for several species, e.g., bull (Röpke et al. 2011), dog (Belala et al. 2016), buffalo (Kumar et al. 2015), and stallion (Pillet et al. 2012).

The fertility of bovine semen stored at 5°C in such diluents remains acceptable for 2 to 4 days (Foote et al. 1960), although that of ram semen only persists for 12 to 24 hours (Evans & Maxwell 1987). The decline in fertility that occurs after this time is initially due to decreased motility and survival in the female genital tract rather than to the death of spermatozoa per se. Short-term storage of semen by chilling to 5°C is, however, a very cheap and effective way of establishing an AI programme for cattle and is of value for on-farm collection and insemination of sheep, whereas the use of liquid boar semen at ambient temperatures remains, effectively, the basis of the technique in that species. Short-term 5°C storage is also widely used in the horse and the dog as it avoids the unpredictable response to freezing that characterises the semen of these species.

Cryopreservation and Cryoprotectants

Longer term storage of semen is achieved through cryopreservation, which facilitates dissemination of desirable genetic material to most parts of the world. Semen doses can be quarantined until the male has been shown to be free of disease at the time of semen collection. Cryopreservation maintains the fertile life of spermatozoa virtually indefinitely, although a large proportion of individual spermatozoa fail to survive the considerable stresses of freezing and thawing. To survive freezing, an extender is used that contains not only substances that protect against cold shock but also cryoprotectants such as glycerol (Polge et al. 1949), which protect them from the deleterious consequences of ice crystal formation.

The general responses of cells to freezing (reviewed by Farrant 1980, Watson 1990) were not understood until long after empirical methods of cryopreservation had become widely adopted. Initially, as the temperature of the external medium falls below its freezing point, crystals of pure water start to form. The concentration of solutes in the unfrozen part of the medium therefore rises as does its osmotic pressure. Ice crystals do not extend into the cell at this stage as they are excluded by the cell membrane. Thus the intracellular contents undergo a period of supercooling, during which the cell loses water to the unfrozen part of the extracellular

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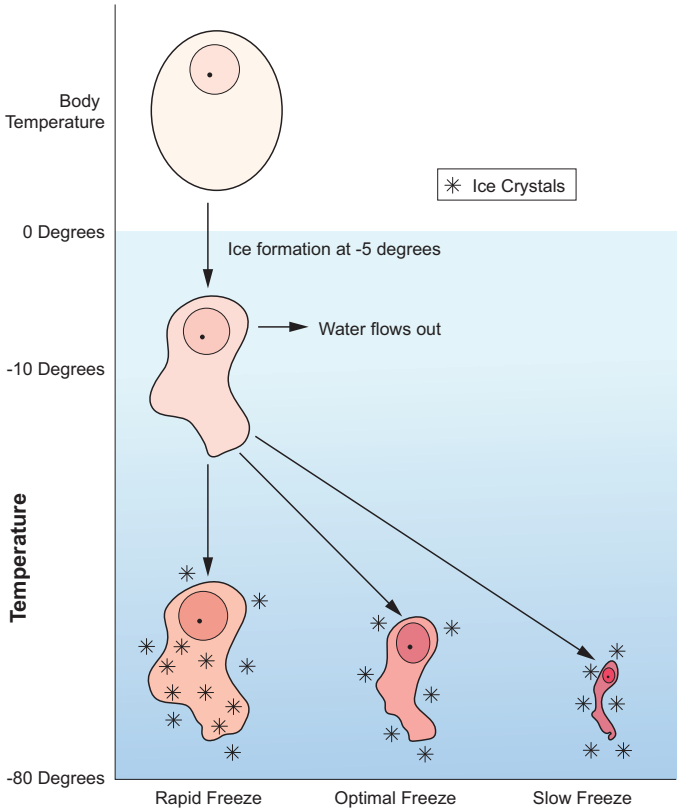

• **Fig. 43.1** Shrinkage of cells during cryopreservation. Extracellular freezing induces conditions that allow osmotically induced loss of water from cells during slow freezing. This correlates with survival on thawing. Rapidly cooled cells do not have time to shrink, form intracellular ice, and are dead on thawing.

medium by osmosis (Fig. 43.1). A variable degree of cell dehydration follows, which is terminated by the formation of intracellular ice crystals. Thus damage can occur to cells in one of two ways. When a substantial degree of cellular dehydration occurs, the high concentrations of solutes in the residual intracellular water can be damaging, whereas, if only slight dehydration occurs, large ice crystals can form within the cell, which cause physical damage to its internal and bounding membranes. The degree to which each affects the cell is determined by the size of the cell and the rate of cooling. Thus the larger the cell, the slower its inherent rate of dehydration; the slower the rate, the more dehydration; the faster the rate, the greater the damage by ice formation.

However, the effects of freezing and thawing also need to be explained in terms beyond those of osmotic effects and ice crystal damage. Spermatozoal membranes are unusual in terms of the lipids of which they are composed and of the arrangements of lipids within the membranes. Cooling and cryopreservation causes phase changes in membrane lipids, which results in loss of integrity, loss of control of calcium movement, and loss of membrane ATPase activity (Holt 2000). Many spermatozoa do not survive these processes – typically less than 50% of the viable spermatozoa that were present before cryopreservation. Moreover, even for the spermatozoa that do survive, capacitation-like changes to the head occur (Curry 2000, Thomas et al. 2006), which alter the duration of their survival in the female reproductive tract. It has been suggested that species-to-species variations in the ability of spermatozoa to survive cryopreservation, as well as some sire-to-sire variability within species, can be attributed to the ability of membranes to withstand or accommodate these changes.

Responses of spermatozoa to cryopreservation can be improved by the use of cryoprotectants, the composition of the extender and the rate at which spermatozoa are frozen and thawed. Cryoprotective agents are classified as those that enter the cell (permeating) or that are retained in the extracellular fluids (non-permeating). The actions of cryoprotectants were originally thought to be mediated solely through hydrogen-bonding of water, thereby reducing the availability of water either for dehydration or for ice crystal formation. Penetrating cryoprotectants (e.g., glycerol or dimethyl sulphoxide (DMSO)) reduce the loss of water from the cell, thereby reducing solute damage, and bind it in a form that renders it unavailable for ice crystal formation. Non-penetrating cryoprotectants, such as disaccharides or proteins, may hasten dehydration during very rapid cooling, thereby minimising intracellular ice formation.

However, other mechanisms are also involved. Polar molecules such as glycerol and sugars appear to form hydrogen bonds with the polar head groups of membrane lipids (Vishwanath & Shannon 2000), stabilising the membranes during transition through critical temperature zones (Woelders 1997). Likewise, insertion of glycerol into the membrane bilayer also appears to affect stability and water permeability of the membranes through altering the physical properties of the membrane lipids (Holt 2000). Sugars such as glycerol alter the mechanical properties of the diluent by increasing its viscosity. This prevents crystallisation of solutes and increases the glass-forming tendency of the medium (a property that is exploited in vitrification methods of freezing larger cells; Isachenko et al. 2004).

Unfortunately, permeating cryoprotectants are relatively toxic to spermatozoa. Glycerol, which is the main primary cryoprotectant used in freezing mammalian sperm, has direct toxic effects upon spermatozoa (Watson 1979, 1990). Concentrations of glycerol that can be tolerated by spermatozoa depend on the species and the other components of the diluent. For example, extenders for bovine semen that contain disaccharides can use lower percentages (3%–4%) of glycerol than diluents that lack such disaccharides, which have a final glycerol concentration of at least 7% (Unal et al. 1978). Bulls' spermatozoa seem to be relatively resistant to the toxic effects of glycerol, whereas boars' spermatozoa, for example, tolerate much lower concentrations before toxic effects start to occur.

Whether the toxic effects of glycerol are exacerbated at high temperatures has been a matter of debate. Early studies (Polge 1953) showed that the addition of glycerol at 28°C was more damaging to bovine spermatozoa than its addition at 4°C, although Salisbury et al. (1978), reviewing the (by then) copious literature, concluded that the effects of temperature were equivocal. It was subsequently suggested that multistep addition of glycerol avoids excessive changes in cell volume that can result in membrane rupture (Gao et al. 1993, 1995). Nevertheless, normal practice in commercial bovine AI centres is that, when the final concentration of glycerol is high (≈7%), a primary dilution of the semen is made with a diluent containing little or no glycerol, with glycerolisation being carried out after reducing the temperature to 4°C. However, diluents that use lower final concentrations (< 5%) are added in one step at 30°C. With boar semen, the toxicity of glycerol at high temperatures is much less equivocal, and low-temperature glycerolisation is desirable (Paquignon 1985). It is possible that the spermatozoa from individual sires may show less tolerance to glycerol than others.

The potential toxicity of glycerol has led to the search for other, less toxic, cryoprotectants. Whereas substances such as ethylene

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glycol and propanediol can be used to cryopreserve embryos, they do not have any advantage over glycerol for freezing spermatozoa. Various amides, however, have been shown to be beneficial, at least for the semen of some individuals and depending on which cryoextender is used as the base (see review by Alvarenga et al. 2005). A combination of dimethylformamide and glycerol in INRA96 was a useful cryoprotectant for stallion semen (Alvarez et al. 2014). In contrast, dimethylacetamide did not improve the survival of boars' spermatozoa (Yang et al. 2016).

Oxidative damage also occurs to the membranes of spermatozoa during the freezing process. A number of studies have implicated membrane lipid peroxidation as a cause of defective spermatozoal function after cryopreservation (Salamon & Maxwell 1995). Attempts to overcome this problem have included the addition of antioxidants such as glutathione peroxidase (Slaweta & Laskowska 1987) or catalase (Shannon 1972), although it has also been suggested that the measurement of the degree of oxidation of membrane phospholipids may be a useful means of assessing the quality of frozen–thawed spermatozoa (Neild et al. 2005).

The first methods for freezing semen involved placing diluted semen in glass ampoules for freezing in a mixture of alcohol and solid carbon dioxide at −79°C, or drops of diluted semen were placed directly on to the surface of a block of solid carbon dioxide where they froze in pellet form (Salisbury et al. 1978). Long-term storage at −79°C was not satisfactory, however, as deterioration occurred at that temperature (Pickett et al. 1961, Stewart 1964). Storage in liquid nitrogen at −196°C has subsequently become established as the standard method for long-term preservation of semen and, over the 40 years for which it has been practised, has maintained semen fertility unscathed. At the present time, semen is frozen in the vapour of liquid nitrogen and then stored long-term in liquid nitrogen. Diluted semen is packed into thin, plastic tubes ('straws' or 'paillettes') of 0.25 or 0.5 mL capacity, then in the simpler techniques, these tubes are suspended in the vapour of liquid nitrogen, which is at about −120°C, for about 10 minutes (Cassou 1964, Jondet 1964). The straws are then plunged into the liquid nitrogen. Subsequently, it was found that a greater degree of control of freezing rate could be achieved by the use of microprocessor-controlled freezers. Some studies have suggested that improved survival of spermatozoa may be sufficient to justify the increased cost of the processing (e.g., Landa & Almquist 1979, Parkinson & Whitfield 1987). On the other hand, although the control of freezing rate is not as good in open tank methods as in programmable freezers, the latter are not as amenable to processing large batches of semen, or if large batchers are processed, there can be significant variation in cooling rate (and hence post-thaw survival) across the cooling chamber. Hence many organisations have chosen to continue to use the less well-controlled open tank methods (Vishwanath & Shannon 2000). Boar semen may be frozen in larger volumes of 5 mL in flat packs (Eriksson et al. 2002).

New Methods of Cryopreservation

Vitrification, a process in which a liquid turns into a solid without the formation of ice crystals, has been evaluated for cryopreservation of cell types that do not survive traditional freezing methods particularly well. Spermatozoa are particularly sensitive to ice crystal formation because their long tail is likely to span regions in the freezing diluent that contain both ice crystals and hyperosmotic diluent (Holt 2000). These random patterns of extracellular ice, together with the intracellular ice that can also be formed during the cooling process, are a significant source of cell damage. In freezing large cells, such as embryos or oocytes, vitrification processes normally require high concentrations of cryoprotectants, which themselves impede cell survival through their toxic effects (Vajta et al. 1997; Vajta 2000). However, recent studies have suggested that ultrafast freezing of spermatozoa can result in vitrification without the presence of a cryoprotectant (Isachenko et al. 2004) and, providing ice recrystallisation during thawing is prevented, results in acceptable post-thaw recovery rates. As yet, it has not been possible to recover motile spermatozoa post-vitrification. However, offspring have been produced after intracytoplasmic injection of reconstituted vitrified spermatozoa (Sánchez et al. 2011, Isachenko et al. 2012), leading to the hope that refinements in the procedure may eventually lead to the retention of spermatozoal motility.

A further new technique is the development of a 'multithermal gradient' as a means of controlling and optimising the formation of ice during the freezing process (Arav et al. 2002). Samples are moved at a constant velocity through a linear temperature gradient (Gacitua & Arav 2005), which controls the point at which ice forms and the morphological characteristics of the ice crystals. This method appears to result in good post-thaw recovery rates (ranging from 45% in the boar to 65% in the bull; Arav et al. 2002) and has the advantage that semen can be frozen in bulk, rather than necessitating the small volumes of pellets or paillettes (Gacitua & Arav 2005).

Thawing

Thawing of the semen needs to be rapid, since slow thawing allows recrystallisation of ice within the cells, causing membrane damage (Salisbury et al. 1978). In practice, the rate of thawing is rarely critical, although thawing at 30° to 37°C for 10 to 60 seconds is commonly recommended for bovine semen (see Fig. 43.3B). Much higher temperatures (e.g., 60°C) for a short period of time have sometimes been recommended as a means of improving recovery rates; however, the practical difficulties of using such methods in the field have meant that they have not been widely adopted. Of far greater importance is the temperature control of the thawed semen, which should not be allowed to cool lower than the final temperature achieved during thawing; otherwise, substantial losses of spermatozoa can occur. Rethawed spermatozoa are as sensitive to fluctuations in temperature as are their unfrozen counterparts (Roberts 1986) and cold shock can easily be induced if post-thaw cooling occurs (Nebel 2007). In addition, controlling the temperature of semen during withdrawal from storage flasks is important (see Fig. 43.3A): temperatures in the top third of the neck of the flask are high enough for ice recrystallisation to occur in the straws, resulting in markedly reduced post-thaw survival (Nebel 2007).

Ambient Temperature Preservation

For spermatozoa to be stored at ambient temperatures, alternatives to cooling have to be found to slow down their metabolic rate. There may also be advantages to ambient temperature preservation of semen because both cooling and cryopreservation are associated with a significant loss of both numbers and functionality of sperm. Moreover, some species do not tolerate cooling or cryopreservation well (e.g., the boar), so for these species, ambient temperature dilution is imperative. Strategies that have been used for ambient temperature diluents include:

- Decreasing the proportion of egg yolk to a level at which the beneficial effects on cold shock are retained but the toxic effects of other yolk components are avoided;

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- Lowering pH to approximately 5.5, resulting in a reversible inhibition of spermatozoal motility (although reducing the pH below 5.5 is spermicidal);
- Saturating the diluent with carbon dioxide also causes a reversible inhibition of motility; diluents, such as the Illinois Variable Temperature (IVT) diluent, that were based on this principle were able to maintain the viability of spermatozoa for approximately 48 hours; longer term maintenance (up to 5 days) is achieved in diluents (e.g., the Cornell University Extender (CUE) or Caprogen diluent) that are self-carbonating from the reaction of citrate and bicarbonate (Vishwanath & Shannon 2000);
- Gassing with nitrogen; this reduces oxygen tension in the diluent, thereby inhibiting the motility of spermatozoa without the need to lower pH; this is used in the Caprogen diluent; and
- A combination of buffers, glucose, and antibiotics (Beltsville Thawing solution) sufficient to maintain boar spermatozoa for several days; longer term storage (up to 7 days) requires a more complex mixture of buffers and also additional antibiotics.

Other components of ambient temperature diluents (simple sugars, buffers, egg yolk/milk, antibiotics, catalase) are similar to those used in diluents for cooled or cryopreserved semen.

Microencapsulation

Encapsulation has also been investigated as a means of preserving spermatozoa without the need for recourse to cryopreservation. Sperm encapsulation is the process of enclosing spermatozoa within a semipermeable membrane (Nebel et al. 1985). Spermatozoa remain viable within the microcapsules because the semipermeable membrane permits the exchange of low molecular–weight nutrients and metabolites with the medium within which they are contained (Roca et al. 2006a), but prevents transfer of large molecules.

The process of microencapsulation has been described by Nebel et al. (1993). After extension in an ambient temperature diluent, spermatozoa are suspended in a sodium alginate solution. Spraying into a high calcium buffer solidifies droplets of diluted sperm, after which the semipermeable membrane is constructed on the surface of the droplet. Polyamines such as protamine sulphate, polyvinylamine, and poly L-lysine have been used successfully. Calcium is then chelated, which converts the gel core of the microcapsule back to a liquid phase. Barium has been used as an alternative to calcium, with the advantage that it does not precipitate premature capacitation (Torre et al. 2007).

Microencapsulation has been used for the ram (Maxwell et al. 1996), the boar (Faustini et al. 2004), and the bull (Vishwanath et al. 1997) semen. Spermatozoa are released from the capsules over a period of at least 24 hours, and in the case of the bull, this may be extended to 96 hours. Preliminary results show that acceptable conception rates can be achieved with encapsulated spermatozoa and that the timing of insemination in relation to the time of ovulation is less critical than with other forms of preserved semen. However, limited development of the technology has taken place for use in insemination, although it is more widely used for other forms of cell preservation (Torre et al. 2007). One such development is to use a solid gel network to immobilise the spermatozoa instead of enclosing them in gel microcapsules to mimic the conditions in the epididymis (Standerholen et al. 2015). Insemination trials in cattle indicate that 56-day non-return rates were similar between conventional frozen thawed semen doses (72.5%) and frozen–thawed, gel-immobilised doses (72.7%) (Standerholen et al. 2015).

The possibility of encapsulating sexed spermatozoa as an attempt to compensate for low spermatozoa numbers is of interest. Use of encapsulated sexed boar spermatozoa reduced the incidence of polyspermy in *in vitro* fertilisation (IVF) and did not produce any additional damage to the spermatozoa during storage for 72 hours (Spinaci et al. 2013).

Spermatozoa Selection

It is possible to improve semen quality by selecting functional spermatozoa from a semen sample. Various methods have been proposed over several decades, as reviewed by Morrell and Rodriguez-Martinez (2016), but only colloid centrifugation of stallion spermatozoa has proved to be practical in the field. Basically, these methods mimic the selection for 'normal' spermatozoa that is believed to occur in the female reproductive tract (Suarez 2007) and can therefore be described as 'biomimetic' (Morrell & Rodriguez-Martinez 2009). These methods rely on one or more physical attributes of functional spermatozoa, namely that they are motile and have normal morphology as well as intact plasma and acrosomal membranes. Thus migration techniques such as 'swim-up' rely on motile spermatozoa being able to move away from an area containing many spermatozoa to one containing relatively few. Passage through Sephadex gel, glass wool, or colloids impedes spermatozoa with reacted acrosomes, damaged membranes, or abnormal morphology. Colloid centrifugation also separates spermatozoa with mature chromatin from those with immature or damaged chromatin.

Colloid-Selected Spermatozoa

Colloids consist of nano-sized particles in suspension; both silica colloids and iodixanol are suitable for the preparation of spermatozoa. Initially, polyvinylpyrrolidone-coated silica was used in density gradients to prepare human spermatozoa for assisted reproduction (Bolton & Braude 1984), and it was also used in this form to prepare animal spermatozoa for IVF. There was some concern that this type of coated silica was toxic to bull spermatozoa (Avery & Greve 1995), but this issue has since been resolved by replacing the PVP-coated silica with silane-coated silica. From 2008, silane-coated silica has been used as a single layer instead of a density gradient (single layer centrifugation; SLC), thus facilitating scaling up of the method to process whole animal ejaculates (Morrell et al. 2009a), even from boars (Morrell et al. 2011). Such SLC-selected spermatozoa samples contain a higher proportion of morphologically normal spermatozoa, which have good motility, intact membranes and good chromatin integrity, than unselected controls (Morrell et al. 2009b). Selected stallion spermatozoa samples survive longer during cooled storage and also show enhanced cryosurvival (Hoogewijs et al. 2011, Martinez-Alborcia et al. 2012); pregnancy rates after insemination are better than controls (Morrell et al. 2014).

There has been some debate whether the apparently enhanced spermatozoa quality after colloid centrifugation is due to selection for a particular spermatozoa subpopulation, or merely to exclusion of immotile or damaged spermatozoa. There is some evidence that the spermatozoa that pass through the colloid produce less hydrogen peroxide as a by-product of metabolism than controls (Morrell et al. 2017), lending credence to the theory that there is selection for a particular spermatozoa subpopulation. However, further research is needed to verify this suggestion. Whatever the mechanism of action, the use of good quality spermatozoa samples for assisted reproduction is associated with enhanced fertility, and therefore this method of processing semen samples is worth pursuing.

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Sexed Semen

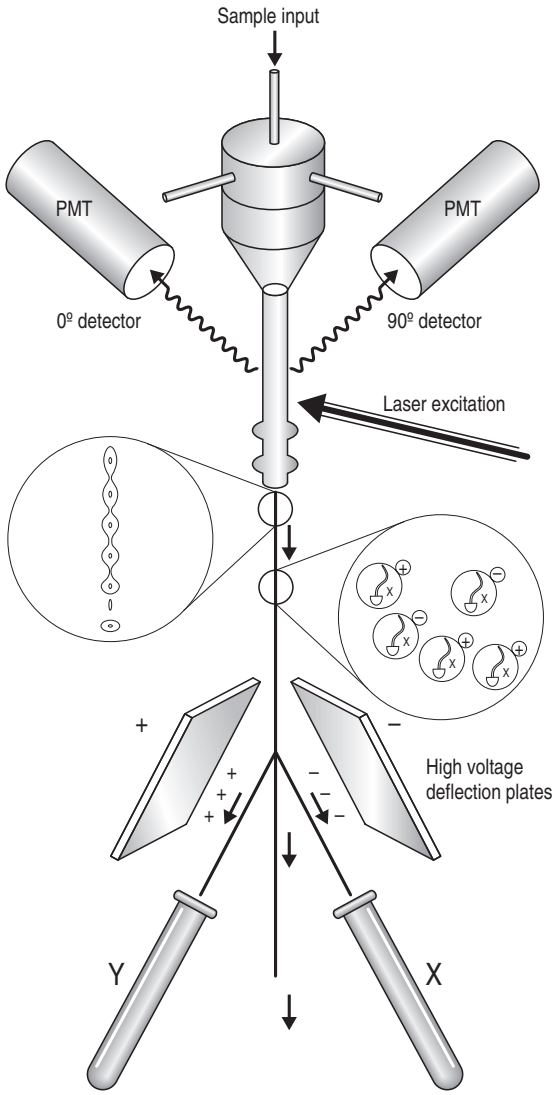
A long-standing goal of the AI industries has been to be able to control the sex of offspring, by separating X- and Y-bearing sperm. Many methods have been developed in attempts to achieve this goal. Most of these proved impractical or ineffective, but in recent years, flow cytometry has been developed to a point at which spermatozoa sexing is available as a commercial service. The technique of sexing semen has been reviewed by Seidel and Garner (2002), Garner (2006), and Seidel (2007).

Separation of spermatozoa on the basis of surface antigens is an attractive option, as it would allow bulk processing of semen. However, early attempts, based on the H-Y antigen, and more recent investigations, based on sex-specific proteins, have yet to yield practical sorting methods. Major problems with this approach are the limited transcription that takes place in the haploid cell and the cytoplasmic bridges that join spermatozoa until just before their release from the seminiferous epithelium. Electrophoresis methods based on the principle that X- and Y-bearing spermatozoa may have different surface charges have also only had limited success to date.

Hence most attention has been upon the using the different mass of DNA in the X- and Y-bearing spermatozoa as the basis for sorting and separation: X-bearing spermatozoa of domestic animals contain approximately 4% more DNA than Y-bearing sperm. The principle of sexing semen is to stain the DNA with a fluorescent dye that binds to the DNA but does not damage it. The difference in fluorescence between the X- and Y-bearing spermatozoa can then be measured and cells sorted accordingly (Keeler et al. 1983, Johnson et al. 1987). DNA is stained with the membrane-permeable agent bisbenzimidazole DNA-binding dye, Hoechst 33342 (Morrell et al. 1988, Garner 2006), which binds specifically to A-T pairs along the minor groove of the DNA (Seidel & Garner 2002). Spermatozoa are separated from live cells by further staining with a food dye, FD&C#40, which permeates dead cells and quenches the fluorescence of Hoechst 33342 but is excluded from living cells. The stained cells are then passed through a flow cytometer for sorting, based on fluorescence from the stained DNA.

After measuring the amount of DNA, the vibrating nozzle in the flow cytometer breaks the solution containing the spermatozoa into tiny droplets, most of which contain a single spermatozoon (some droplets contain no sperm, a few contain two sperm). A charge is placed on the droplet as it leaves the sorter such that droplets containing spermatozoa with Y chromosomes are given a negative charge and those containing spermatozoa with an X chromosome are given a positive charge. The droplets then pass through an electromagnetic field, which deflects droplets with a positive charge (X chromosomes) towards the negative side (and vice versa for negatively charged droplets). Somewhere in the region of 20% of spermatozoa are successfully sorted as X-bearing, 20% as Y-bearing, with the remainder unidentified or dead. This process is illustrated in Fig. 43.2.

The process is relatively slow, however. Currently, 14 insemination doses of 2×10^6 live spermatozoa of each sex can be produced per hour (Seidel 2014). Moreover, the spermatozoa have been subjected to a number of insults during the separation process (staining, laser, high pressure, electrical charge), which impair their subsequent function. There is evidence of more chromatin abnormalities in sorted spermatozoa (Bochenek et al. 2006), but H33342 does not appear to cause any mutagenic damage to the genome. Altering the conditions for the cell sorting process can reduce the overall level of spermatozoa losses (Schenk & Seidel 2007). Recent developments by the patent holders have apparently improved the sex-sorting conditions such that conception rates are now similar between nonsexed and sexed spermatozoa doses (Lenz et al. 2016). However, no details of the process were provided. It remains to be seen whether similar results can be obtained in field AI under conventional conditions.



• **Fig. 43.2** Flow cytometric sorting of X- and Y-bearing sperm. After staining, the sperm are passed by photomultiplier tubes (PMT). The electronic signals from the PMT are sent to a computer for analysis. Thereafter the fluid stream is broken into droplets and a positive charge is placed on droplets identified to contain a Y-bearing sperm and negative charge on droplets with X-bearing sperm. The drops then travel between high voltage fields, which direct them to their respective collection tubes. (Derived from Johnson & Welch 1999, Seidel & Garner 2002.)

Sorted spermatozoa can survive cryopreservation, but the low numbers of spermatozoa that are present generally require specialised insemination routes to achieve acceptable pregnancy rates (see later in this chapter). Alternatively, sexed semen can be used in *in vitro* fertilisation (cattle) or intracytoplasmic spermatozoa injection (horse) for the production of embryos of known sex (Wheeler et al. 2006). However, as noted by Garner (2006), the effective use of sexed semen in animal breeding industries requires further development in the speed and efficiency of sex sorting, as

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TABLE 43.1 Diseases that may be transmitted through artificial insemination

	Cattle	Sheep	Pigs	Horse
Present and Spread Through Semen	Bovine herpesvirus 1 Bovine immunodeficiency-like virus Bovine venereal campylobacteriosis Brucellosis Contagious bovine pleuropneumonia Epizootic haemorrhagic disease Foot & mouth disease *Histophilus somni* Japanese encephalitis Johnes' disease Mycoplasma spp Trichomonosis Tuberculosis Vesicular stomatitis	Bluetongue Contagious caprine pleuropneumonia Foot & mouth disease Japanese encephalitis Johnes' disease Lentivirus (maedi-visna) Ovine enzootic abortion Ovine epididymitis (*Brucella ovis*) Ovine/caprine brucellosis Peste de petits ruminants Sheep pox/goat pox Vesicular stomatitis	African swine fever Aujeszky's disease Classical swine fever Foot & mouth disease Japanese encephalitis Leptospirosis Porcine brucellosis Reproductive and respiratory syndrome Swine vesicular disease	African horse sickness Dourine Equine viral arteritis Venezuelan equine encephalomyelitis Vesicular stomatitis
Low Risk of Spread	Akabane virus Bluetongue Bovine ephemeral fever Bovine virus diarrhoea Enzootic bovine leucosis Leptospirosis	Caprine arthritis/encephalitis Leptospirosis		Contagious equine metritis Equine herpesvirus 3 (coital exanthema) Piroplasmosis Equine infectious anaemia
Reference	Eaglesome & Garcia 1997, OIE 2016	Cseh et al. 2012, OIE 2016	Althouse & Rossow 2011, OIE 2016	Metcalf 2001, OIE 2016

well as development in the technology of AI in order to utilise such spermatozoa efficiently. The idea of combining encapsulation and sex-sorting, as previously mentioned, may represent one such development (Spinaci et al. 2013).

Diseases Transmissible in Semen

Many infectious agents can be transmitted through semen. Foot and mouth virus can be transmitted in the semen of all species that are susceptible to this infection (Callis & McKercher 1980, Radostits et al. 2007). Indeed, control of foot and mouth transmission has, until recently, underpinned the UK legislation controlling AI of cattle, with broadly similar regulations in force throughout much of the developed world. Diseases of cattle, small ruminants, and horses that are potentially transmissible in semen are listed in Table 43.1.

The World Organisation for Animal Health (OIE) therefore stipulates that sires that are to be used in AI centres for international trade should be tested for freedom from a number of diseases. Many importing countries also require animals to be free of additional diseases. When semen is diluted in egg yolk, there is generally a requirement that the eggs are derived from specific-pathogen-free (SPF) flocks. These regulations are also applied to use of semen in domestic trade within the European Union.

Artificial Insemination of Cattle

Collection and Storage of Semen

Semen is usually collected by an artificial vagina, although electroejaculation is occasionally used (see Chapter 35). After assessment for motility, the concentration of spermatozoa, and, occasionally, their morphology, the semen is extended into insemination doses. The degree of extension and the extenders used depend on its intended application. Most bull semen is cryopreserved, although some is used after simple extension and chilling to 4°C. The practice of using an ambient temperature extender for bull semen is virtually unique to New Zealand, where the practice is made possible by the low incidence of infectious disease, and is required for the highly seasonal pattern of reproduction in its dairy cows.

For cryopreservation or for use at 4°C, the semen is first extended with an extender based on either egg yolk or skimmed milk (Salisbury et al. 1978, Vishwanath & Shannon 2000); see Table 43.2. The semen is then cooled to the appropriate storage temperature. If it is destined for use cooled, spermatozoal motility will be reassessed before releasing the semen for use. If the semen is destined for cryopreservation, glycerol is also added; then the semen is packed into 0.25 or 0.5 mL paillettes (or, rarely, 0.5 or 1.0 mL glass ampoules). The semen is then 'equilibrated' for 1 to 4 hours. It was originally considered that this was the period in which glycerol penetrated the sperm, although subsequent observations indicate that the penetration of glycerol is very rapid and that most of the equilibration period is concerned with membrane stabilisation during exposure to low temperatures (Watson 1979). Recently, there has been a trend to equilibrating the semen in cryoprotectant overnight before freezing (Anzar et al. 2011), which may be more compatible with the routines on the bull station. The semen is then frozen in liquid nitrogen vapour or in a microprocessor-controlled freezer. The semen thereafter remains in liquid nitrogen until thawed for use. Freezing in alcohol and solid carbon dioxide or in pellets on blocks of solid carbon dioxide, although formerly used widely, has now virtually ceased.

The ability to perform an intrauterine insemination in cattle means that a relatively low dose of spermatozoa is required to achieve acceptable pregnancy rates. Typically, of the 10 to 25 × 10^6 spermatozoa that are required in each insemination dose, 6

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TABLE 43.2 Diluents for use in cryopreservation of bovine semen

Constituent	Skimmed Milk	Egg Yolk–Citrate	Reading Diluent	Egg Yolk–Tris	Netherlands Extender
Egg yolk	100 mL	200 mL	200 mL	200 mL	200 mL
UHT skimmed milk	870 mL				
Fructose	12.5 g				
Lactose			82.8 g		
2.9% sodium citrate buffer[a]		770 mL			
Tris buffer				800 mL[b]	800 mL[c]
Citrate–HCO_3 buffer					753 mL
Glycerol			47 mL		47 mL
Stage 1:	30 mL	30 mL		Nil	
Stage 2:	110 mL	110 mL		140 mL	
Antibiotics	Typically, 1000 IU penicillin + 1000 µg streptomycin/mL				

[a]Trisodium citrate dihydride.
[b]30.28 g Tris, 17.30 g/litre citric acid monohydrate.
[c]31.5 g Tris, 12.3 g/litre citric acid monohydrate.
Stage 1 diluents are added at 30° to 37°C, after which the semen is cooled to 4°C. Stage 2 diluents are added at 4°C. Single stage diluents are added at 30° to 37°C, after which the semen is cooled to 4°C.
From: Salisbury et al. 1978, Parkinson & Whitfield 1987, Vishwanath & Shannon 2000, J. Wilmington, personal communication.

to 7×10^6 survive freezing, a figure that is generally regarded as the minimum dose compatible with acceptable fertility (Milk Marketing Board 1967, Sullivan & Elliott 1968). An exception is when sexed semen doses are used because the sexing procedure takes such a long time: typically 1.5 to 2.0×10^6 spermatozoa are inseminated, although pregnancy rates tend to be 80% of those obtained with unsexed spermatozoa (Garner 2006). Lower numbers of spermatozoa can be used for fresh semen (Salisbury & VanDemark 1961).

The use of ambient temperature extenders for bull semen is precluded in most countries by the risk of contamination of the semen with foot-and-mouth disease virus. However, it has many advantages over other methods of extension because many more spermatozoa survive in the absence of freezing, albeit for a limited time. Whereas cryopreserved bull semen requires about 10 to 25×10^6 spermatozoa per insemination, semen extended in an ambient temperature extender can achieve acceptable fertility with fewer than 2.5×10^6 spermatozoa per insemination (Shannon et al. 1984). Initial work on ambient temperature diluents for bovine semen was based upon the use of the IVT diluent (Salisbury & VanDemark 1961, Melrose 1962), but the development of ambient temperature dilution has subsequently been based upon the use of the CUE and Caprogen diluents (Shannon 1965; Table 43.3). With the subsequent modifications that have taken place, these diluents are now capable of maintaining spermatozoa viability and acceptable conception rates for up to 5 days, with an insemination dose of between 0.5 and 2.5×10^6 spermatozoa.

TABLE 43.3 Diluents for ambient temperature extension of bovine semen

Basic Diluent (g/Litre)	Cornell University Extender	Caprogen Diluent
Sodium citrate	14.5	20
Citric acid	0.87	
Sodium bicarbonate	2.1	
Glycine	20	10
Glycerol		12.5
Glucose	3	3
Potassium chloride	0.4	
Caproic acid		0.3125
Preparation of Diluent		
Basic diluent	80%	80%
Egg yolk	20%	20%
Nitrogen gas		Bubbled for 20 min at 5°C
Catalase		4.5 mg/L

Derived from: Shannon 1965, Shannon & Curson 1984, Vishwanath & Shannon 2000.

Insemination

Before insemination, straws of semen are withdrawn from liquid nitrogen storage, ensuring that the canister containing the frozen semen remains contained within the neck of the storage flask (Fig. 43.3A). After identification of the sire, excess liquid nitrogen

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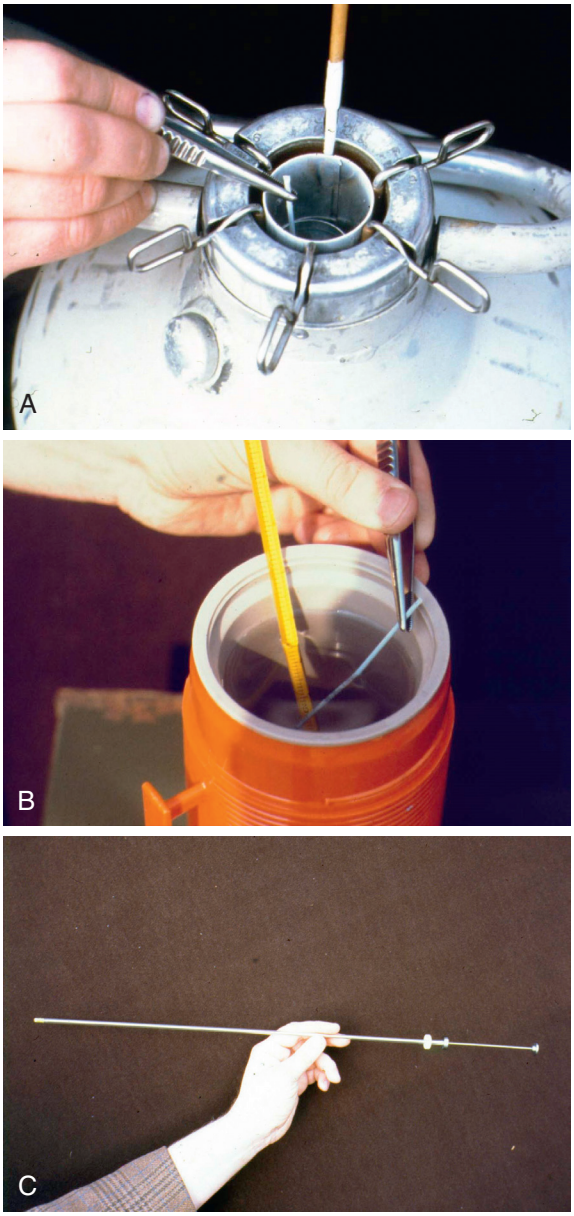
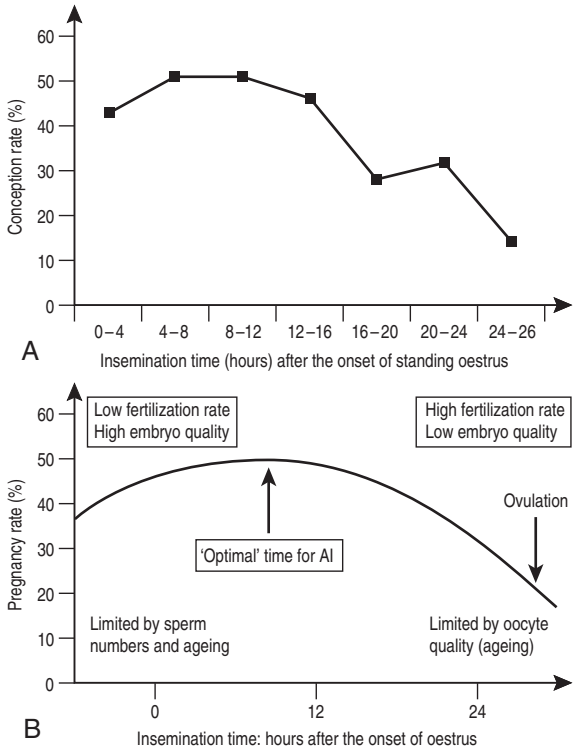


• **Fig. 43.3** Semen handling for bovine artificial insemination. (A) Withdrawing a straw of frozen semen from the liquid nitrogen flask. The canister containing the semen should not be lifted higher than the level of the top of the neck of the flask. (B) Thawing. After checking the identity of the sire, the straw is thawed. Water temperature is not really critical but placing the straw in water at 37°C for 10 seconds is a typical thawing regime. (C) The straw is placed in an insemination catheter, which is then covered with a plastic sheath. The catheter is then ready for use, but care must be exercised not to allow the semen to become chilled again before it is inseminated.

is flicked out of the end of the straw before it is deposited into thawing water (Fig. 43.3B). After thawing, the straw is dried and the identity of the sire is rechecked, before loading it into the insemination catheter (Fig. 43.3C). It is important that the temperature remains constant after thawing, as semen must not be allowed to cool lower than the temperature at which it was removed from the thawing water. Hence, it is generally preferable to thaw small numbers of straws at a time, depending on the proficiency of the inseminator and the temperature of the environment.



• **Fig. 43.4** Optimal timing of insemination in cattle (A) derived from observations of the onset of oestrus; (B) as a compromise between fertilisation rate and embryo survival rate. (Redrawn from Saacke et al. 2000.)

Timing

Cows ovulate approximately 12 hours after the end of oestrus. The ideal time for insemination is therefore 6 to 24 hours before ovulation (Roberts 1986). Observations of conception rates of animals that were inseminated at different times after oestrus (Fig. 43.4A) led to the development of the 'a.m.–p.m.' rule, by which cows first seen in heat in the morning were inseminated that afternoon, whereas those first seen in heat in the afternoon were inseminated the next morning (Olds & Sheath 1954, Foote 1979).

In fact the optimal timing of insemination is a compromise between many factors. Insemination early in oestrus is likely to achieve a low fertilisation rate because of limitations on spermatozoal viability, whereas oocytes that are successfully fertilised are likely to yield high quality embryos. Conversely, insemination late in oestrus is less likely to result in limitations on spermatozoa viability but is more likely to result in the fertilisation of an aged oocyte, resulting in impaired embryo development (Saacke et al. 2000). According to this proposal, the 'optimum' timing of insemination represents a compromise between the processes of aging of spermatozoon and oocyte (Fig. 43.4B). However, these processes are also affected by the duration of oestrus and the interval between the onset of oestrus and ovulation as well as the degree to which the spermatozoa have been affected by the preservation process. The latter can further involve both precapacitation changes and impairment of spermatozoa viability in the female tract.

Site

Standard Method of Insemination

Cows are inseminated at the internal cervical os or just inside the short uterine body. The standard technique of insemination is to grasp the cervix through the rectum with the left hand. A

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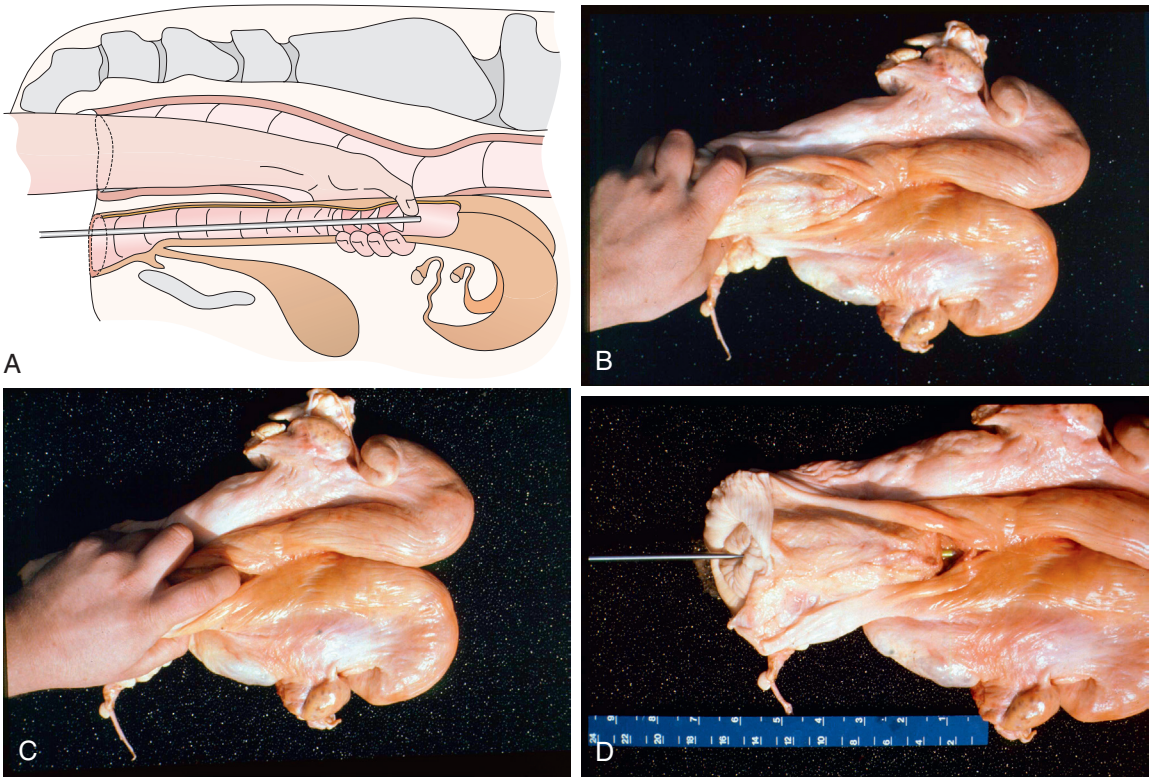

• **Fig. 43.5** Rectovaginal method of insemination. (A) General method. (B) Partially dissected bovine uterus showing grip on the cervix for the obliteration of the vaginal fornix to facilitate entry of the catheter into the external os of the cervix. (C) Second grip on the cervix to allow passage of the catheter through the tortuous cervical canal: the index finger is gently pressed over the internal os in order to feel the tip of the catheter as it emerges from the cervical canal. (D) Site of deposition of semen in the uterine body: the recommended site is no more than 0.5 cm deep to the internal cervical os.

catheter, into which a paillette of semen has been inserted (Figs 43.3C, 43.5A) is then passed into the vagina and manipulated into and through the cervix by the right hand. This technique, the rectovaginal method of insemination, requires considerable practice for success. The vulval lips are opened by downwards pressure from the arm in the rectum, while the circular folds of vaginal mucosa are obliterated by pushing the cervix forward. The catheter is initially inserted pointing upwards at an angle of about 30° to avoid entering the urethral meatus or fossa and is then moved horizontally until it engages in the external os of the cervix. The left hand squeezes the anterior vagina on to the caudally projecting external os of the cervix, thereby obliterating the fornix of the vagina (Fig. 43.5B) and facilitating entry of the catheter into the cervix. Entry into the external os is accompanied by a characteristic 'gritty' sensation. The catheter is then introduced through the convoluted cervical canal by manipulation of the cervix through the rectal wall. One finger is placed over the internal os of the cervix so that the tip of the catheter can be palpated as it emerges from the cervical canal (Fig. 43.5C). As soon as the catheter has emerged, deposition of semen into the uterus begins and the catheter is not advanced any deeper into the uterus. In this way, semen should be equally distributed between the two uterine horns (Figs 43.5D and 43.6A, B). Insemination deeper into the uterus runs the risks of either inseminating into the uterine horn contralateral to the ovulation site or scoring the endometrium with the tip of the insemination catheter. Reduced fertility is the consequence of these errors.

No forward pressure should be exerted on the catheter with the right hand because the uterine wall is friable and easily penetrated if the catheter moves suddenly. The most common fault of insemination (Fig. 43.7A) is twisting the cervix in the left hand, so that one uterine horn is partly occluded. Alternatively, the catheter may be partly withdrawn during the deposition of semen, resulting in a partial intracervical insemination (Fig. 43.7B). Penetration of the cervical canal of maiden cattle is difficult at oestrus and virtually impossible at other stages of the oestrous cycle. Such animals are therefore often beyond the capabilities of inexperienced inseminators; it is common to find that semen is deposited into the caudal part of the cervix. However, the cervix of parous cattle can be traversed relatively easily at most stages of the oestrous cycle and early pregnancy. It is therefore imperative to know whether an animal is likely to be pregnant before insemination is attempted, for abortion can be induced if an insemination catheter penetrates the fetal membranes or if infection is introduced into a pregnant uterus by poor insemination hygiene.

Alternative Sites for Insemination

Although traditional methods of insemination avoid deep uterine deposition of semen in cattle, this has been advocated as a means of improving conception rates (Senger et al. 1988, Dalton et al. 1999). However, this has not been achieved in all studies (e.g., McKenna et al. 1990) and has not been widely adopted in practice. Single horn insemination has also been advocated as a means of improving conception rates in cows. This technique requires

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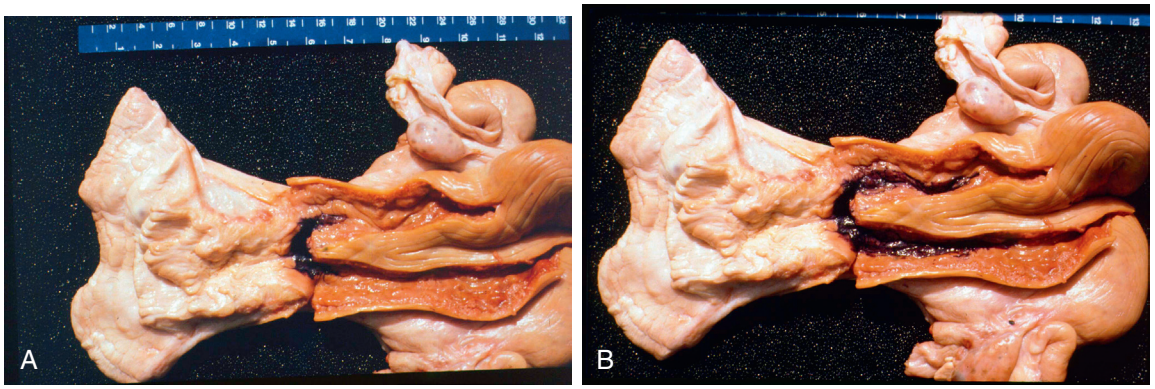
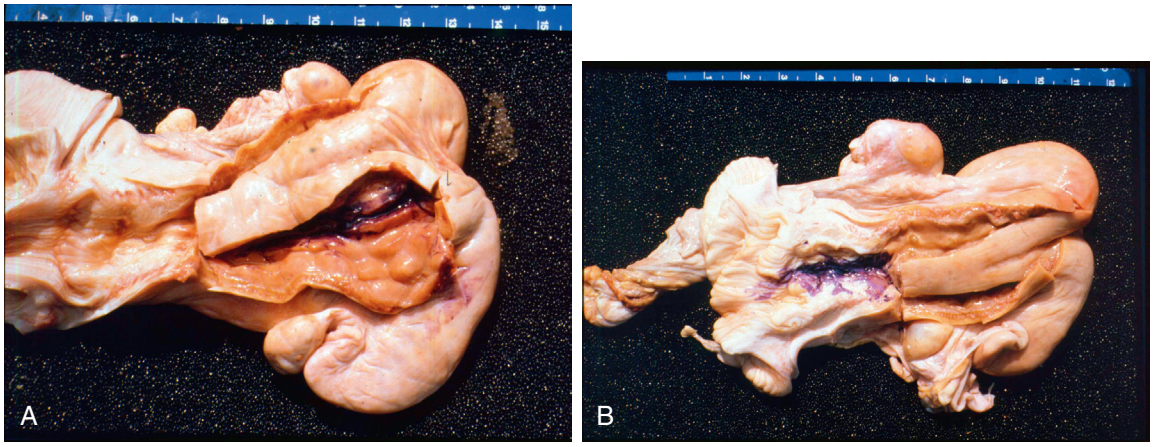


• **Fig. 43.6** Distribution of semen in the cow after artificial insemination. A dark-coloured dye has been placed into the uterine body (A) and runs equally into both uterine horns (B). As inseminators are trained not to palpate the uterus or ovaries, they should be unaware of the side on which ovulation will occur, so semen must have access to both uterine horns.



• **Fig. 43.7** Distribution of dye in the reproductive tract of the cow after faulty insemination. (A) Uterus twisted during insemination, occluding one uterine horn. The dye is present in only one horn, typically the right. (B) Catheter withdrawn into the cervix during semen deposition. Some dye is present in the uterine body, but most is within the cervical canal.

palpation of the ovaries to determine the site of ovulation and a careful straightening of the ipsilateral horn to allow the insemination catheter to be inserted to within approximately 2 cm of the uterotubal junction. Again, some authors have found that this technique results in improved conception rates (Pallares et al. 1986, Lopez-Gatius & Camon-Urgel 1988), whereas others have not (Hawk & Tanabe 1986, Momont et al. 1989). Disadvantages include premature rupture of the follicle, perforation of the uterine wall, risk of polyspermic fertilisations (Hunter 2003), and possibly also an increase in the time required to perform the insemination.

Despite the equivocal results in field trials, some continue to advocate the use of deep intrauterine insemination, especially in situations in which semen may be compromised, as for example, in bulls whose semen does not respond well to preservation or when sexed semen is used (Hunter 2000, Lopez-Gatius 2000).

Management of Insemination

Inseminations may be performed by technicians employed by AI organisations but increasingly is being done by farm staff.

Fertility after AI is generally monitored by recording the proportion of cows that return to oestrus after the initial service. The proportion of females that do not return to oestrus is sufficiently closely related to the proportion that actually have become and remained pregnant to be a useful monitor of fertility (reviewed by Salisbury et al. 1978). The figure thus obtained, the non-return rate (NRR), which is an overestimate of calving rate, is generally in a fixed ratio to the calving rate depending on the interval after initial insemination at which it is calculated. The NRR is useful for observing trends in performance and for identifying below average performance. The NRR can be used by AI centres to monitor both the fertility of their bulls and the results obtained by their technicians. Technicians whose performance is below average are normally required to undergo a period of retraining and/or limited licensure until their results improve. Bulls that produce consistently low figures are removed from the AI service. Some centres make use of an 'adjusted NRR', whereby a number of factors that may affect fertility, such as cow or heifer, age of female, number of parities, location of farm, and identity of inseminator, are taken into account to create a 'fertility index score'. This score is believed to give a more accurate picture of a bull's fertility than using the unadjusted NRR.

Farm staff can be trained to inseminate cattle, with many countries allowing 'farmer-inseminators' to breed their own cattle. There are two potential advantages that persuade farmers to undertake

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their own inseminations: first, the avoidance of the costs associated with having an AI centre technician perform the insemination; and, second, the hope that improved timing of insemination to ovulation will raise conception rates. Morton (2000) undertook a widespread survey of the results achieved by farmer-inseminators in comparison to AI centre technicians. Overall, farmer-inseminators achieved a 3% lower conception rate than centre technicians (45% vs 48%). However, although 13% of farmer-inseminators achieved conception rates that were more than 5% higher than centre technicians, 45% had results that were more than 5% worse, and 12% had results that were more than 15% worse than centre technicians.

In practice, farmer-inseminators who are well trained, motivated, have enough time to perform inseminations, and have reasonably large herds tend to achieve good results, whereas those for whom it is 'another job' or who do not have the opportunity to develop a good insemination technique do not. Poor insemination technique can be associated with disastrously poor pregnancy rates.

Control of Infectious Diseases

Many diseases can be spread in bovine semen (Table 43.1); AI centre technicians, by travelling between farms, also pose a considerable risk as disease vectors. Consequently, there is legislative control over the health status of semen used in domestic trade, whereas international trade in bovine semen is subject to the requirements and recommendations of the OIE. There are also controls over the hygienic practices of inseminators. Diseases that are specifically regulated by the OIE are listed in Table 43.4.

Most of the serious viral diseases of cattle (particularly foot-and-mouth disease) can potentially be transmitted through AI. Legislative regulation of bovine AI has for many years been based upon the primary precept of preventing such transmission from occurring. Many other viral diseases, such as bovine herpesvirus-1, can also be transmitted in this manner (Chapman et al. 1979, Kahrs et al. 1980). Bovine viral diarrhoea (BVD) virus can be present in the semen of bulls (Barlow et al. 1986), potentially causing early embryonic death and abortions in inseminated cows (Grahn et al. 1984), as well as its better known ability to cause the birth of persistently infected progeny. Recently, it has become apparent that Schmallenberg virus can be transmitted in semen (Schulz et al. 2014). Other viral diseases that could be of potential concern to bovine AI include Akabane, ephemeral fever, and bovine immunodeficiency viruses. However, Thibier and Guerin (2000) reviewed literature suggesting that Akabane virus has not been detected in semen; moreover, although ephemeral fever and bovine immunodeficiency virus may be present in bull semen, transmission by AI has not been demonstrated. There is no evidence that the prion agent responsible for bovine spongiform encephalopathy (BSE) can be transmitted through semen (Wrathall 1997, Wrathall et al. 2008).

A number of bacterial diseases are transmissible in semen, including tuberculosis and brucellosis (Eaglesome & Garcia 1997). *Histophilus somni,* pathological species of *Mycoplasma* and *Ureaplasma diversum* may also be present in semen (Humphrey et al. 1982). Most importantly, the classic venereal pathogens of cattle, *T. foetus* and *C. fetus* subsp. *venerealis* are transmissible by AI, so control of these two organisms remains the second major precept upon which legislation governing cattle AI is based.

Three other conditions warrant specific mention. Firstly, although enzootic bovine leukosis (EBL) virus is considered not to be transmissible in semen (Radostits et al. 2007), infected bulls are excluded from AI studs. Secondly, bluetongue virus generally causes few or no clinical signs in infected cattle (Radostits et al. 2007), but cattle can act as sources of infection for other animals via arthropod hosts. Because the disease in sheep is severe, semen from potentially infected bulls (Bowen & Howard 1984) is carefully excluded from most countries in which sheep production is of economic importance. Finally, Johne's disease is also commonly regulated, as semen from clinical cases and subclinically infected animals is potentially contaminated with the organism.

In cattle, control of these diseases rests upon four major strategies:

i. Direct effects of freezing and thawing: *Leptospira* spp. may be killed by the freezing process.
ii. Antibiotics that are added to semen diluents in order to kill both pathogenical bacteria (including *C. fetus*) and the contaminant bacteria that originate from the penis and prepuce during semen. Antibiotics are also used to control mycoplasma and ureaplasma species.
iii. Serology: Diseases that can be detected by serology, such as brucellosis, IBR, EBL, Q fever, are controlled by exclusion of seropositive bulls from AI studs. Likewise, tuberculosis is controlled by exclusion of bulls that react to tuberculin testing. However, a negative serology test result only indicates that the animal has not reacted to an infection by producing antibodies; recent infections or congenital carriers may never exhibit an antibody response.
iv. Quarantine: The final and most potent means of control of disease is the quarantine of semen after its collection. After semen has been frozen, it is placed in a container where it remains untouched for 28 days. If, during that period, the donor bull develops any disease, the semen is destroyed. If not, it is released for use.

TABLE 43.4 World Organisation for Animal Health requirements for disease testing of sires use of semen in artificial insemination programmes

Cattle	Sheep and Goats	Pigs
Bluetongue	Bluetongue	African swine fever
Bovine herpesvirus-1	Brucellosis	Aujeszky's disease
Bovine viral diarrhoea	Caprine arthritis/encephalitis	Brucellosis
Brucellosis	Contagious agalactia	Classical swine fever
Campylobacter fetus subsp. *venerealis*	Contagious caprine pleuropneumonia	Enterovirus encephalomyelitis
Tritrichomonas foetus	Lentivirus (maedi-visna)	Porcine reproductive and respiratory syndrome
Tuberculosis	Ovine epididymitis	Transmissible gastroenteritis
	Paratuberculosis	Tuberculosis
	Peste des petits ruminants	
	Scrapie	
	Tuberculosis (goats)	

From: OIE 2016.

Artificial Insemination of Sheep

Artificial insemination of sheep has been comprehensively reviewed by Evans and Maxwell (1987), Chemineau et al. (1991), and Kukovics et al. (2011). The sheep is less amenable to artificial insemination than is the cow because oestrus cannot be detected readily

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TABLE 43.5 Minimum numbers of motile spermatozoa for insemination of ewes at different sites

Route	Number of Motile Sperm (x 10^6)			Insemination Volume	Concentration of Inseminate (x 10^6/mL)
	Fresh	Liquid	Frozen		
Vaginal	300	400		0.3–0.5 mL	2000
Intracervical	100	150	180	0.05–0.2 mL	1000
Transcervical intrauterine		60		0.1–0.5 mL	200–400
Laparoscopic intrauterine	20	20	20	0.05–0.10 mL per horn	400–800

Derived from: Evans & Maxwell 1987, Usboko 1995, Shipley et al. 2007.

without the presence of rams, the technique of insemination is less straightforward than in cows, and ovine semen is less easy to freeze than bovine semen. In Eastern Europe, South America, and Australasia, AI is widely used in sheep breeding programmes, but its use is much less widespread in Western Europe and North America, mainly because of the high costs of handling and inseminating sheep compared with the costs of natural service. Fertility after AI depends on breed (whether dairy or meat breeds) and other factors such as parity, climate, farm, technique, and inseminator (Anel et al. 2005, Palacin et al. 2012).

In contrast to cows, ewes normally display oestrous behaviour only in the presence of a ram. In order to determine the time at which AI should be performed, either the timing of oestrus should be controlled or it should be detected with rams. In the former situation, pharmacological methods are used to induce and synchronise oestrus, so that the time of the fertile period is defined. In the latter situation, raddled, vasectomised rams are used to detect oestrus. The cost:benefit ratio for the use of AI in sheep has therefore to be considered carefully. When there are substantial costs associated with AI, such as either maintaining rams, or of buying and administering drugs for oestrus synchronisation, or of the procedures associated with insemination itself, these have to be set against the financial benefits gained from the superior carcass or wool characteristics of the progeny born to AI.

However, the most important limitation of the use of AI in sheep is in the method of insemination, as intrauterine insemination is difficult to achieve because the cervical canal of the ewe is so tortuous. Because intracervical AI results in both a lower conception rate and a lower number of lambs per litter than natural service, a number of methods of insemination have been devised that try to bypass the cervix. All of these methods have had varying success in achieving an economically viable compromise between fertility, technical difficulty, and numbers of spermatozoa needed for insemination. The methods currently in widespread use are via the intravaginal, intracervical, transcervical intrauterine, and laparoscopic intrauterine routes.

Collection and Storage of Semen

Semen is collected by an artificial vagina or by electroejaculation (see Chapter 35) and is subjected to routine examination for motility and spermatozoa concentration. Most inseminations of ewes are performed using semen that has been extended by the addition of simple extenders, because the small volume of the ovine ejaculate means that it is difficult to control spermatozoa numbers if used for direct insemination. Extender and the final number and concentrations of spermatozoa depend on the route of insemination, whether the insemination is undertaken during the natural breeding season or after induction of out-of-season breeding, and how the semen is to be stored between collection and insemination (i.e., direct insemination, cooled storage, cryopreservation). Recommendations for spermatozoa numbers and insemination volume are shown in Table 43.5.

TABLE 43.6 Diluents for ram semen for use at 5° to 15°C

Constituent (g/L)	Tris Diluent	Egg Yolk–Citrate	Skimmed Milk
Tris	36.3		
Fructose		5.0	
Glucose	5.0		10.0[a]
Citric acid	19.9		
Sodium citrate		23.7	
Skimmed milk powder			90.0
Egg yolk (mL/L)	140	150	50.0
Antibiotics	1000 IU penicillin and 1000 µg streptomycin/mL		

[a]Recommended by Feredean et al. 1967 for addition to the basic skimmed milk diluent.
Derived from: Evans & Maxwell 1987, Salamon & Maxwell 2000.

Liquid Semen

Extenders that are in routine use for unfrozen semen include buffers containing citrate or Tris, together with either egg yolk or cow's milk (Table 43.6). When milk is used, it should be heated first to destroy the spermicidal lactenin protein, so either skimmed or UHT-treated milk is used. After dilution, the semen is cooled and stored at either +15°C or +4°C until used. The semen should be used within 8 hours of collection, as fertility declines progressively after this time (Chemineau et al. 1991), with a 30% reduction in lambing rates after storage for 24 hours (Maxwell & Salamon 1993). More recently, Quan et al. (2016) suggested that the motility of spermatozoa was preserved for longer in Tris– or Tes–egg yolk extenders than in skimmed milk.

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Cryopreservation

Although simple extenders are capable of supporting spermatozoal viability for the relatively brief periods demanded for direct insemination, cryopreservation is required for long-term storage of semen. Unfortunately, cryopreservation of ovine semen is not particularly straightforward because the process itself causes a significant level of damage to ovine spermatozoa. In consequence, frozen semen is not recommended for intravaginal insemination, and although it can be used for intracervical insemination, fertility results are relatively poor.

There are also a number of difficulties encountered when semen is frozen for intracervical insemination. These arise as a relatively large number of spermatozoa ($150–200 \times 10^6$ total sperm; Salamon & Robinson 1962, Langford & Marcus 1982) have to be contained within the limited volume of inseminate that can be placed within the ovine cervix. Because the anatomy of the cervical canal limits the insemination volume to below about 0.25 mL (Evans & Maxwell 1987), the dilution rates are limited to between 1 : 1 and 1 : 4. In consequence, insufficient protection can be afforded to the spermatozoa by the diluent against cold shock and freezing damage (Miller 1986), generally resulting in mediocre postfreezing survival of functional sperm.

For intrauterine insemination, in which lower numbers of spermatozoa are required, far more satisfactory dilution rates of semen can be achieved, so cryopreservation is more successful. Furthermore, because the insemination site does not require spermatozoa to traverse the cervix, they do not have to survive in such a robust state as is needed for intracervical insemination. Hence the conception rates that are achieved with the use of cryopreserved semen for intrauterine insemination are commensurate with those of natural service after oestrus synchronisation (see, for example, Davis et al. 1984).

Extenders

Early extenders for cryopreservation of ram semen were based on citrate, egg yolk, a monosaccharide such as glucose or fructose, and/or milk. Other cryoextenders have been based on disaccharides (e.g., lactose), trisaccharides (e.g., raffinose), complex polysaccharides (e.g., gum arabic), or other complex molecules (e.g., polyvinylpyrrolidone). Optimal glycerol concentrations seem to be in the range of 4% to 6% (Salamon & Maxwell 2000), although this concentration is affected by other components of the extender, such as egg yolk and sugars. Although early studies suggested that there might be advantages to adding glycerol at 5°C, or to its stepwise addition, current practice is to add the glycerol at approximately 30°C. Two diluents that are currently widely recommended for ram semen are Tris–glucose–egg yolk (Salamon & Visser 1972) and Tris–citrate–fructose–egg yolk (Shipley et al. 2007) (Table 43.7), although there are many other diluents that are used successfully in commercial or research practice.

The problem of low dilution rates for semen that is to be used for intracervical insemination was addressed by Salamon (see Evans & Maxwell 1987) by preparing a range of different extenders for freezing semen at ratios of 1 : 1 to 1 : 4, in which the constituents were present in higher concentrations. In this way, compensation is achieved for the effects of the ratio between extender and seminal plasma. Chemineau et al. (1991) dealt with the same problem by using two different extenders (an egg yolk–lactose diluent and a glycerolised skimmed milk diluent) in varying proportions depending on the final dilution rate to be achieved.

One of the possibilities for improving the response to cryopreservation was the inclusion of seminal plasma in the extender.

TABLE 43.7 Diluents for the cryopreservation of ram semen

Constituent	Tris–Glucose	Tris–Citrate–Fructose
Basic Diluent		
Tris (g/L)	36.3	24.4
Fructose (g/L)		10.0
Glucose (g/L)	5.0	
Citric acid (g/L)	19.9	13.6
Glycerol (mL/L)	50.0	64.0
Preparation of Diluent		
Basic diluent	85%	80%
Egg yolk	15%	20%

Derived from: Evans & Maxwell 1987, Shipley et al. 2007.

Evidence reviewed by Maxwell et al. (2007) suggests that it is generally beneficial in terms of post-thaw membrane integrity and motility. Low molecular weight proteins (15–25 kDa) appear to be most associated with these benefits, and it has been postulated that a spermadhesin protein may be the critical component. As yet, however, the inclusion of seminal plasma has not been incorporated into field practice.

More recently, attention has focused on the inclusion of specific lipids such as oleic acid (Hashem et al. 2017) and palmitoleic acid (Eslami et al. 2017) into extenders. Bashawat et al. (2016) found that the low density of lipoprotein gave better overall outcomes after freezing than did egg yolk. Fish oil, as a source of docosahexaenoic acid, improved postfreezing recovery (Abdi-Benemar et al. 2015), whereas supplementing the diet with fish oil also improved post-thaw spermatozoal quality, pregnancy rate, and lambing rate (Masoudi et al. 2016a). Soybean lecithin as a source of phospholipid (Paz et al. 2010), specifically phosphatidylcholine (Masoudi et al. 2016b), has been successfully used to replace egg yolk in diluents. Finally, cholesterol, in the form of cholesterol-loaded cyclodextrin, has been used to improve the motility and proportion of intact acrosomes in a skimmed milk diluent (Salmon et al. 2017).

Addition of various antioxidants to semen extenders in an attempt to reduce lipid peroxidation has been increasing in popularity. Thus cysteine (Toker et al. 2016), green tea extract (Mehdipour et al. 2016), and taurine (Banday et al. 2017) were found to have a beneficial effect on post-thaw spermatozoal quality.

Freezing Protocols

After dilution, ram semen should be cooled slowly from 30°C to 5°C, as rapid cooling below 5°C is associated with poor spermatozoal survival (Fiser & Fairfull 1986). Hence cooling to 5°C should take place over 1.5 to 2 hours: Shipley et al (2007) recommend that this is best managed in a water bath. During this period of cooling, equilibration with glycerol takes place. Thereafter, semen can be successfully frozen in pellet form on the surface of blocks of solid carbon dioxide (Salamon 1971) or in paillettes in the vapour of liquid nitrogen (Fiser & Fairfull 1984). There is, however, much variation in the ability of the semen of individual rams to survive cryopreservation and the semen of a proportion of rams does not survive freezing successfully.

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Paillettes are thawed in water, typically at temperatures of 35° to 40°C. Pellets can be thawed in a dry tube in a water bath or can be placed in prewarmed thawing solution. It is suggested that pellets are thawed at 40°C, then maintained in a 30°C water bath until required for insemination (Shipley et al. 2007).

Insemination

Vaginal Route

Vaginal insemination deposits semen into the cranial part of the vagina without attempting to locate the cervix. The requirements of this method, in terms of both technical proficiency and handling facilities for the sheep, are minimal. However, the large numbers of spermatozoa that are required for each insemination means that the vaginal route is not really amenable for use with stored semen. Moreover, conception rates are also poor after pharmacological oestrus synchronisation, so intravaginal insemination is best suited to use after oestrus detection during the natural breeding season. The ideal timing of insemination is before ovulation, i.e., 12 to 18 hours after the onset of oestrus (Evans & Maxwell 1987). Highest conception rates are therefore achieved when the timing of insemination is optimised by drafting ewes for insemination twice per day.

Intracervical Route

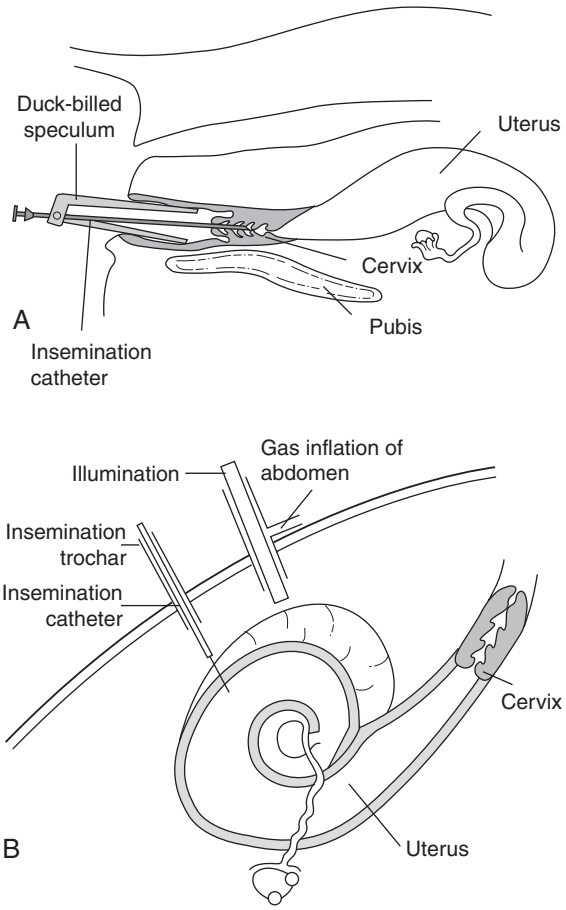
Intracervical insemination is best achieved with the hindquarters of the ewe elevated. After cleaning of the perineum, the vagina is opened with a duck-billed speculum, and the cervix is located (Fig. 43.8A). The insemination catheter is then inserted as far as possible into the cervix; penetration of the cervix is typically 0 to 2 cm.

The conception rate is highly correlated with the depth of penetration (Table 43.8) and hence the technical proficiency of the inseminator. Conception rates achieved with the use of unfrozen semen by this method are adequate after oestrus synchronisation (Chemineau et al. 1991). The ideal time for insemination is 55 ± 1 hours after removal of intravaginal progesterone inserts or 15 to 17 hours after the onset of detected oestrus. However, even when substantial numbers of cryopreserved spermatozoa are used for intracervical insemination, conception rates are below those of natural service or fresh semen insemination (Colas 1979). Conception rates of 65% to 80% are typical when this method is used in the ewe's breeding season, with a lower figure for out-of-season breeding regimens. According to Kukovics et al. (2011), pregnancy rates of 80% to 90% were achieved on Hungarian sheep farms using fresh semen inseminated via this route.

Laparoscopic Intrauterine Route

The method of direct intrauterine, laparoscopic insemination (Fig. 43.8B) was developed to overcome many of the difficulties of intravaginal and intracervical insemination. In this method, ewes are sedated and restrained in a cradle. Laparoscopy is performed close to the udder after administering local anaesthetic. The abdomen is insufflated with CO_2, the uterus is located and semen is injected into the uterine lumen via a small stab wound (Killeen & Caffery 1982). The semen can be introduced to the uterus via a simple pipette (Evans & Maxwell 1987) or by the use of specialised insemination equipment (Chemineau et al. 1991). With oestrus-synchronised ewes, the ideal timing of insemination is between 48 and 65 hours after withdrawal of progesterone sponges, with conception rates declining after 72 hours postwithdrawal (Salamon & Maxwell 2000). Conception rates to frozen semen inseminated by this method are higher than for intracervical insemination because of better cryopreservation of spermatozoa and a site of insemination that avoids spermatozoa having to traverse the cervix. Conversely, the laparoscopy is technically demanding, and there are far greater implications for the welfare of the ewes than with intracervical insemination.

The development of laparoscopic intrauterine insemination of ewes is undoubtedly the most significant development in sheep AI in recent years, for it circumvents many of the problems of traditional methods (Haresign et al. 1986). The number of spermatozoa required for each insemination is lower and the volume of inseminate is proportionally greater, allowing more appropriate



• **Fig. 43.8** Insemination of the ewe: (A) intracervical route. (B) Laparoscopic intrauterine insemination.

TABLE 43.8 Lambing rates (%) in relation to the depth of intracervical insemination

No. Spermatozoa Per Inseminate ($\times 10^6$)	Site of Insemination: On the Entrance Fold of the Cervix	Up to 1 cm into the Cervix	Deeper than 1 cm into the Cervix
400 (undiluted)	50	68.8	71.4
100 (diluted)	43.8	66.7	71.4
50 (diluted)	25.7	60.7	66.7

From: Evans & Maxwell 1987.

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dilution rates and therefore better preservation of sperm. Hence conception rates are similar to those achieved by natural service, and embryonic mortality is reduced to an acceptable level. The method also allows for the possibility of genuine progeny testing of rams, as semen from an individual sire can be used in many flocks over prolonged periods of time. Consequently, laparoscopic AI has been adopted in most of the major sheep producing countries (Haresign 1992). In Israel, a pregnancy rate of 71.4% was achieved with this method (Alfaris et al. 2012).

Transcervical Route

Transcervical insemination is a new method that attempts to achieve an intrauterine insemination via the cervical route. In this method the cervix is fixed by being grasped with forceps, and an insemination needle is introduced as far as possible through its lumen. Although better conception rates can be achieved by this method than by conventional intracervical insemination (Souza et al. 1994), both the retraction of the cervix and the penetration of the cervical lumen are associated with significant levels of trauma (Usboko 1995, Campbell et al. 1996). It is probable that, with the development of expertise in the method, its use will become more widespread, but at present, it seems to have little to commend it over laparoscopic insemination. In fact, pregnancy rates may be lower than those achieved with other methods even when no cervical trauma is evident (Wulster-Radcliffe et al. 2004).

Conception Rates to AI

Conception rates depend on the route and timing of insemination, whether ewes are being bred at synchronised or spontaneous oestrus, and whether fresh or frozen semen is used (Table 43.9). Equally importantly, it also depends on the management and condition of the ewes and the care with which the AI programme is managed. Results may also be difficult to interpret due to confusion in the terminology used, e.g., whether transcervical is really intracervical or not.

Control of Infectious Diseases

Diseases of sheep and goats that are transmissible in semen and that are regulated by OIE are listed in Table 43.4. *Brucella melitensis* and *B. abortus* can both be transmitted in semen, and of the organisms responsible for ovine epididymitis, *B. ovis* can be transmitted in semen; the pleomorphic Gram-negative organisms are also excreted in semen. Other significant diseases of sheep and goats that are controlled in AI donor sires include maedi-visna, caprine arthritis encephalitis virus, sheep pox, and goat pox, although there is no clear evidence for their transmission through semen (Thibier & Guerin 2000). It does not appear likely that the prion responsible for scrapie can be transmitted through this route (Wrathal 1997).

TABLE 43.9 Typical lambing rates achieved via different routes of insemination using fresh or frozen semen

Site of Insemination	Semen	Number of Motile Sperm	Lambing Rate (%)
Intravaginal	Fresh	400 x 10^6	20–60
	Frozen		5–20
Intracervical	Fresh	200 x 10^6	40–80
Synchronised oestrus	Frozen		25–40
Spontaneous oestrus	Frozen		30–60
Laparoscopic intrauterine	Fresh	20 x 10^6	70–95
	Frozen		40–80
Transcervical	Fresh	200 x 10^6	40–80
	Frozen		30–70

Derived from: Shipley et al. 2007.

Artificial Insemination of Goats

Artificial insemination of goats is generally very similar to that of sheep; many of the factors affecting pregnancy rates in sheep are also relevant for goats (Abecia et al. 2016). However, it is much easier to achieve an intrauterine insemination via the cervix of the goat than that of the ewe. Second, although oestrus detection is more efficient in the presence of a buck (or after a synchronisation protocol), it is possible to detect it in the absence of a buck (Nuti 2007). Consequently, there has been a significant uptake of AI as a means of breeding goats in both the commercial and lifestyle sectors. In France, for example, the numbers of goat inseminations increased fourfold during the 1980s (Chemineau et al. 1991). On the other hand, there are difficulties in preserving buck semen, whether cooled or cryopreserved, because of toxic interactions between seminal plasma and milk/egg yolk-based extenders.

Collection and Storage of Semen

Semen is generally collected using an artificial vagina (AV). It can be collected by electroejaculation, but this is less well tolerated than in the ram. Because electroejaculation affects the composition of seminal plasma, it can reduce the ability of spermatozoa to survive preservation (Leboeuf et al. 2000). Bucks are seasonal breeders with a decrease in the quality and quantity of semen produced in the spring and summer. This decline can be mitigated if bucks continue to copulate regularly throughout the year (Corteel 1981).

Effects of Seminal Plasma upon Storage

The major problem with the preservation of goat semen is that there are components in seminal plasma that impair the viability of spermatozoa stored in media containing milk and egg yolk. The toxic interaction with egg yolk is due to an egg yolk–coagulating enzyme (EYCE) that is secreted by the bulbourethral gland. This enzyme coagulates egg yolk and hydrolyses lecithin to fatty acids and spermicidal lysolecithins (Iritani & Nishikawa 1964). It is considered that the primary activity of EYCE is via a phospholipase A, but it may also contain lipase (Leboeuf et al. 2000). Bulbourethral gland secretions also have a toxic interaction with milk. A 55 to 60 kDa glycoprotein lipase, originally named SBUIII (Nunes et al. 1982), now called BUSgp60 (Pellicer-Rubio et al. 1997), which is structurally related to pancreatic lipase PLRP2, releases oleic acid from milk triglyceride (Pellicer-Rubio & Combarnous 1998). Skimmed milk can therefore be used as a diluent, although its effectiveness depends on the extent to which it contains any residual fat.

As a consequence, extenders for goat semen have either been based on skimmed milk, in which spermatozoa survive adequately, or the seminal plasma has been removed before using egg yolk–based diluents (Corteel 1974). When yolk-based diluents are used, washing the spermatozoa is achieved by dilution in Krebs-Ringer phosphate solution and centrifugation (Corteel & Paquignon 1984). Spermatozoa survive for 12 to 24 h after semen collection.

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Liquid Semen

Two main strategies have developed for the use of liquid semen in goat AI.

Cooled Semen in Simple Diluents

- Removal of seminal plasma by washing in a simple buffer, followed by dilution in egg yolk–citrate or egg yolk–tris–fructose diluent and use at 5°C (Haibel 1986, Evans & Maxwell 1987, Chemineau et al. 1991).
- Because removal of seminal plasma is time-consuming and leads to loss of total numbers of spermatozoa, skimmed milk extenders can be used as an alternative for storage at 5°C. Such extenders are widely used (Leboeuf et al. 2003), although the effective lifespan of spermatozoa in skimmed milk extenders is relatively short (around 12 hours).
- Low levels of inclusion of egg yolk (~2.5%) do not result in the toxic effects of high inclusion rates; glucose citrate or Tris fructose plus a low concentration of egg yolk have also been used effectively (Shamsuddin et al. 2000, Bispo et al. 2012, Jiménez-Rabadán et al. 2012).
- Extenders that do not contain material of animal origin, e.g., soy lecithin (Roof et al. 2012), have been found to be as effective, if not more so, than egg yolk extenders.

Ambient Temperature Diluents

- If spermatozoa are not cooled below 15°C, egg yolk is not needed to prevent cold shock (Leboeuf et al. 2000). Skimmed milk and the commercial, soya–lipid-based diluent Biociphos Plus (IMV, L'Aigle, France) can maintain the viability of spermatozoa at 15° to 22°C for approximately 24 hours (Paulenz et al. 2005).
- Defined diluents, based on the antioxidant TEMPOL (4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl) (Mara et al. 2007) or phosphocaseinate (Leboeuf et al. 2003), have also proved effective.

Cryopreservation

For cryopreservation, egg yolk–based extenders are more widely used than skimmed milk–based ones, although acceptable results can be achieved with skimmed milk (Corteel 1974). The most widely used extenders (Leboeuf et al. 2000) are skimmed milk–glucose (Corteel 1974) and Tris–glucose–citric acid–egg yolk (Salamon & Ritar 1982), although soy lecithin extenders are gaining in popularity (Roof et al. 2012). When the final concentration of egg yolk is kept below 2.0% of the diluent (Ritar & Salamon 1982), egg yolk–based extenders can be used without removal of seminal plasma. Glycerol is the most effective cryoprotectant and can be added in one or two steps. For washed spermatozoa, Chemineau et al. (1991) recommend that primary dilution should be in glycerol-free, egg yolk–citrate diluent, followed by addition of extender containing 14% glycerol once the semen has reached 4°C. However, when unwashed spermatozoa are used, semen can be glycerolised in a single step at 30°C (Ritar & Salamon 1982, 1983).

For intracervical insemination, there are similar problems of numbers of spermatozoa in relation to the volume of inseminate to those that apply in the sheep. Hence, Salamon (Evans & Maxwell 1987) also suggested variable composition extenders (which also contain a final concentration of egg yolk of <2.0%) for one step dilution of goat semen for use in intracervical insemination at dilution rates of between 1 : 1 and 1 : 4.

Semen can be frozen either by the pellet method (i.e., on the surface of solid carbon dioxide) or in paillettes that are suspended over the vapour of liquid nitrogen or used in programmable freezers. Post-thaw recovery rates are better with pellet freezing than with other methods (Purdy 2006), but because acceptable recovery rates are also achieved with paillettes, the convenience of handling semen means that it is usually the preferred method of cryopreservation. Although much variation exists between individual animals, overall results with doses of 50×10^6 motile frozen–thawed spermatozoa inseminated into the uterus are comparable with those of natural service (Ritar & Salamon 1983). The relationship between numbers of spermatozoa and fertility is not known because few data from large scale surveys on the subject exist. Nevertheless, Haibel (1986) suggested that 100 to 125×10^6 spermatozoa are used per insemination, whereas Evans and Maxwell (1987) recommended similar numbers to those used in sheep. Semen is thawed using similar protocols to those for the ram.

Insemination

Similar routes of insemination can be used in the doe to those employed in the ewe. Intravaginal insemination is effective if fresh semen is directly inseminated (Nuti 2007) but gives poor results with extended or frozen semen. Hence the main means of insemination are intracervical insemination or laparoscopic intrauterine. Intracervical insemination is the most widely practised method, especially in 'lifestyle' herds. In a significant number of does, an intrauterine insemination can be achieved via the intracervical route (50%–60%; Cseh et al. 2012) because the caprine cervix is relatively easier to traverse than the ovine cervix. Interestingly, although intrauterine insemination can be achieved simply by direct pressure of the insemination catheter upon the cervix, many technicians prefer to deposit at least some of the semen into the cervical canal in case the intrauterine insemination had been entirely into a single uterine horn (Haibel 1986). Nuti (2007), however, suggested that the rates of insemination failure after intracervical insemination are relatively high, either because of failure to enter the cervix properly or because of damage to the cervix or uterus during the insemination process. Depth of insemination is related to pregnancy rate: intrauterine insemination results in the highest rate, with the chances of conception declining significantly as the site of deposition becomes further caudal in the cervix (Salvador et al. 2005). Operator proficiency is therefore a critical determinant of pregnancy success. Pregnancy rates as high as 80% are reported for fresh semen.

Laparoscopic intrauterine insemination is performed in the same way as in the ewe. It has the advantages of requiring a lower dose of spermatozoa (~20×10^6) and achieving a higher pregnancy rate than intracervical insemination but is more demanding to perform (Cseh et al. 2012). Sohnrey and Holtz (2005) suggested that deep corneal transcervical insemination produces results that are at least as good as that achieved by laparoscopy.

Artificial Insemination of Pigs

One of the main stimuli to the use of AI in pig breeding is the possibility it confers of maintaining a closed herd by obviating the necessity of introducing purchased boars. Furthermore, because the traits of economic importance in the pig (i.e., carcase conformation, growth rate, and feed conversion efficiency) have high heritability and can be evaluated in the sires themselves, genetic selection can be intense so that the potential value of AI boars in enhancing the genetic base of a breeding herd is considerable.

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Traditionally, the use of AI in pig breeding has been greatest in Eastern Europe and in countries such as Holland and Denmark with very high densities of pigs. However, there has been a marked increase in the use of pig AI in many other countries, in parallel with the improvements in AI technology and the demand for high genetic–merit sires (Johnson et al. 2000). In the UK, only about 9% of the national herd was bred by AI in the survey of Iritani (1980), whereas the figure in 2004 was 60% to 70% (Goss 2004). The United States has seen a similar growth of AI in pigs over the same period (Johnson et al. 2000). In Europe, more than 90% of sows have been bred by AI in the last two decades (Maes et al. 2011). Most inseminations in Europe are performed by farm staff as the costs of a technician service are too great to be economically viable, although technician services are available in some countries.

Two main difficulties exist in pig AI. First, the period of maximum fertility is not particularly easy to detect in sows (Evans & McKenna 1986) so that animals have traditionally had to be inseminated two (or even three) times during oestrus to maximise conception rate and litter size. Second, pregnancy rates and litter size are reported to be lower after AI with frozen semen than with fresh semen (Alm et al. 2006). Hence, insemination of sows has been based upon multiple inseminations of semen that has been extended and stored at 16° to 18°C containing large numbers of spermatozoa (~2.3×10^9). This has meant that each ejaculate produces enough doses to inseminate only 15 to 25 sows (Maes et al. 2011), a smaller number of doses per ejaculate than in other species such as bulls. Reducing the insemination dose to 1.8×10^9 causes a decrease in litter size of 0.07 piglets (Feitsma 2009), which has a marked effect on the economics of pig breeding.

Collection and Storage of Semen

Semen is manually collected from boars in the presence of a dummy sow (see Chapter 35). The frequency of collection depends on the semen characteristics of individual boars, but it is usually twice per week (Vyt et al. 2007). The pre-ejaculatory fluid is discarded, and the postsperm-rich fraction may not be included either, depending on individual practice or whether a sealed collection device is used. Between 100 and 150 mL of sperm-rich fraction is produced, which is filtered to remove gel, and is extended for insemination after evaluation for spermatozoa concentration, motility, and possibly spermatozoa morphology. Previously, when using the 'gloved hand' technique, it was possible to move the collecting vessel into position once it was apparent that the clear spermatozoa-free prostatic portion was changing to the coloured, sperm-rich fraction of the ejaculate, but the availability of sealed collection containers and automatic collection devices means that the whole ejaculate is collected instead. The sealed collection devices are believed to reduce the number of bacteria that contaminate the ejaculate during semen collection.

Liquid Semen

Approximately 99% of pig inseminations are undertaken using liquid semen (Johnson et al. 2000), and because pig spermatozoa are highly sensitive to cold shock, nearly all of these use ambient temperature extenders. When semen is to be stored at ambient temperatures, the activity of spermatozoa has to be inhibited to ensure an adequate period of viability.

Extenders designed to be used at ambient temperature were based upon the IVT extender throughout the 1960s and 1970s (Paquignon 1984). These have more recently been superseded by diluents such as the Guelph (= Kiev) diluent, BTS (Beltsville Thawing Solution) diluent, and Zorlesco diluent (Haeger & Mackle 1971, Pursel & Johnson 1975, Gottardi et al. 1980). The Guelph diluent is simpler than the IVT diluent to prepare but maintains the fertility of semen for a similar length of time. The more complicated Zorlesco diluent may maintain semen fertility for up to 12 days, although field trials indicated that the effective period is probably shorter than that. Newer diluents, such as the Zorpva (Cheng 1988), Reading (Revell & Glossop 1989), and Androhep (Weitze 1990) diluents, have reliably maintained the fertility of spermatozoa over longer periods of up to 5 days and are, consequently, in increasingly widespread use (Almond et al. 1998), although the most widely used diluent in the United States is the BTS (Johnson et al. 2000). Addition of antioxidants, buffering agents, and antibiotics extend the shelf life of the semen doses (Knox et al. 2016). Examples of short- and long-term diluents are given in Table 43.10.

Insemination doses are variously recommended to contain a minimum of 1×10^9 sperm, with increased numbers when semen is not used on the day of collection. Althouse (2007) suggests that, as a rule of thumb, spermatozoa numbers are increased by 1×10^9 for every day of delay between collection and insemination; hence a common dose rate for commercial boar AI services is 2.3×10^9 spermatozoa (Maes et al. 2011). If semen is collected in situations in which spermatozoa concentration cannot be determined accurately or if it is for local use within a breeding establishment, conservative dilution at 1:4-1:7 is recommended (Althouse 2007) to result in 2 to 4.5×10^9 spermatozoa per dose (Almond et al. 1998). The volume of material that is inseminated is important, because low volume insemination fails to achieve acceptable conception rates. Hence, a total insemination volume of 70 to 100 mL (typically 80 mL) is used. The necessity for using such a large volume is probably to stimulate uterine motility in the sow, thereby ensuring that adequate numbers of spermatozoa reach the site of fertilisation. Semen is typically packaged into plastic tubes, bags, flatpacks, or bottles and is maintained above 15°C before insemination.

For AI in commercial piggeries, it is common practice to mix the semen of different boars in insemination doses. This reduces the risk associated with the responses of an individual boar's semen to extension and dilution, making the outcomes of insemination more predictable (Godet et al. 1996). However, it does make the tracking of an individual boar's performance for the elimination of poor performers more difficult.

Cryopreservation

The semen of boars does not generally respond well to cryopreservation. Recovery after thawing is poor and highly variable between individual sires, and fertility of semen is invariably substantially lower than that of extended semen. Typical results for frozen semen are, at best, 70% of sows conceiving to a double insemination, compared with 80% to 90% for liquid semen insemination. Litter size is also lower after frozen than after liquid semen insemination (Roca et al. 2006b). Consequently, only approximately 1% of pig inseminations use frozen semen, which is largely limited to semen that has been traded internationally.

There are two major limitations to success in freezing boar semen. First, it is highly sensitive to cold shock, and neither egg yolk nor skimmed milk provides anything like the degree of protection against cold shock that it provides to the spermatozoa of most other species (Watson 1979). Second, although glycerol is probably the best cryoprotectant for the boar spermatozoa, its toxic effects are more pronounced in this species than in most others (Wilmut & Polge 1974). In consequence, the numbers of spermatozoa that are

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TABLE 43.10 Diluents for ambient temperature preservation of boar semen

	Short Term (Up to 3 Days)		Long Term (3–5 Days)		
Constituent (g/L)	Guelph (Kiev)	BTS	Zorlesco	Zorpva	Androhep
D glucose	60.0	37.0	11.5	11.5	26.0
Disodium EDTA	3.7	1.25	2.3	2.35	2.4
Sodium hydrogen carbonate	1.2	1.25	1.25	1.75	1.2
Trisodium citrate	3.7	6.0	11.7	11.65	8.0
Citric acid			4.1	4.1	
Tris			5.5	5.5	
Hepes					9.0
Polyvinyl alcohol (type II)				1.0	
Bovine serum albumin					2.5
Cysteine			0.1	0.07	
Antibiotics	Lincomycin, spectinomycin, gentamicin, neomycin, and/or benzyl penicillin plus streptomycin are commonly added to suppress mycoplasmas and *Leptospira* spp.				

Derived from: Haeger & Mackle 1971, Evans & McKenna 1986, Cheng 1988, Weitze 1990, Almond et al. 1998, Johnson et al. 2000.

required to obtain even the mediocre conception rates that follow the use of cryopreserved semen are very high indeed: typically 5 to 6×10^9 spermatozoa per insemination dose (Paquignon 1984). Hence, when frozen semen is used, only about five animals can be inseminated per ejaculate. This low extension rate effectively limits the use of frozen semen to international traffic in pig genetics and to the preservation of gene stocks for future use.

A great deal of work has been undertaken to improve the results of cryopreservation of porcine semen, which, despite the unpromising appearance of early studies, has resulted in better and more consistent survival of freezing. Progress has been made in a number of areas (reviewed by Johnson et al. 2000, Roca et al. 2006b).

Improvement in Extenders

The protective effect of egg yolk is greatly improved when detergent (OrvusEs Paste (OEP)) is added to the extender, probably as it breaks down the fat globules and allows better interaction of the egg phospholipids with the membranes.

Addition of antioxidants, such as vitamin E, glutathione, or superoxide dismutase, prevents lipid peroxidation during freezing and thawing. Addition of resveratrol from grapes or epigallocatechin-3-gallate from green tea to the extender had a beneficial effect on post-thaw penetration rates in IVF, even though no effect on spermatozoa viability or acrosome integrity was observed (Gadani et al. 2017). It has been suggested that adding seminal plasma to thawed boar spermatozoa may counteract lipid disorder arising during the cryopreservation process (Torres et al. 2016).

Improvement in Control of Freezing

Temperature control is a critical aspect of the success with which the spermatozoa of any species can be frozen. The large volume straws (5–6 mL) in which pig semen has traditionally been frozen do not provide good control of temperature, either during freezing or during thawing, especially in the centre of the straw. Much better responses are achieved when semen is packaged into 0.5 mL straws in aliquots of approximately 2×10^9 sperm, then diluted after thawing to an appropriate volume for insemination. Alternative 'flat pack' systems of freezing have also been developed, which package up to 5×10^9 spermatozoa in 5 mL of diluent but ensure even cooling throughout the pack. Saravia et al. (2010) reported good cryosurvival in a simplified procedure, freezing spermatozoa from only the first 10 mL of the sperm-rich fraction in 'mini flat-packs', with a considerable shortening of the procedure. As the authors indicate, the remainder of the ejaculate could be used for processing conventional semen doses (Saravia et al. 2010).

Selection of Sires

Boars can be selected for response to the freezing of their semen; selection for 'good' freezers results in more consistent quality of frozen–thawed semen and fewer ejaculates that fail to survive the freezing process. Roca et al. (2006a) even suggested that boar semen should be test frozen for identification of 'good' and 'bad' freezers. In this context, it is interesting to note that, in the early days of bovine AI, a similar selection process was practised, with an effective elimination of 'bad' freezers (at least of dairy breeds) from studs.

Management of Insemination

Spermatozoa that survive freezing have undergone significant capacitation-like changes that reduce the duration of their survival in the female tract. Re-examination of the insemination process with regard to the site of deposition of semen and the timing of insemination (see later in this chapter) is allowing significant improvements in pregnancy rates and litter sizes to cryopreserved semen.

Current Practice

Saravia et al. (2005) summarise the processing of semen for freezing, based on methods described by Eriksson and Rodriguez-Martinez (2000a, b). After holding at room temperature for approximately 1 hour, the semen is extended 1 : 1 in BTS. The extended semen is

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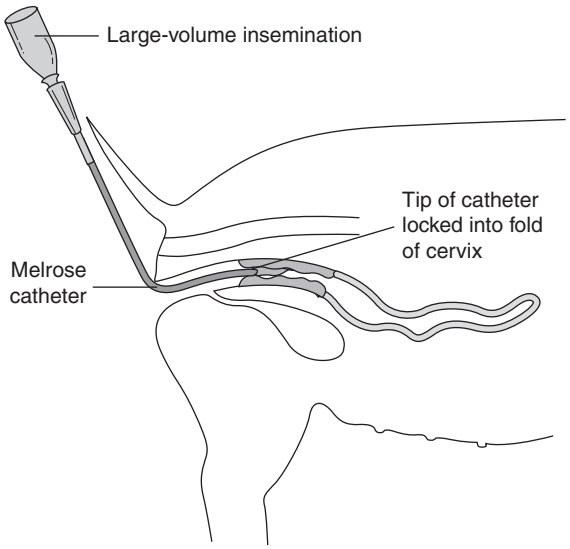
held at 16°C for 3 hours, after which it is centrifuged at 800 g for 10 minutes. After centrifugation, the supernatant is discarded, and the pellets are resuspended in a lactose–egg yolk diluent. The semen is then cooled to 5°C over 2 hours in the centrifuge. Thereafter, semen is mixed with a further lactose–egg yolk extender, which also contains OEP and glycerol (to give a final concentration of 3% glycerol).

After packaging into appropriate containers, the semen is frozen in static liquid nitrogen vapour or in a programmable freezer. The optimal cooling rate over the range 0° to 50°C is generally accepted as being −30°C/min (Johnson et al. 2000). This cooling rate has to be matched by a very rapid thawing rate.

Insemination

The optimum time for AI is during a 24-hour period in the middle of the 50 to 60 hours of oestrus, so that capacitated spermatozoa are present at the time of ovulation. The highest fertility results are achieved when insemination is undertaken 10 to 12 hours before ovulation (Evans & McKenna 1986). However, the interval between the onset of oestrus and ovulation is variable, and even twice daily oestrus detection may not accurately pinpoint the onset of oestrous behaviour. Hence it is recommended that optimal conception rates and litter sizes are achieved by delaying insemination until 12 to 18 hours after the first detection of oestrus, with a second insemination 12 hours (Evans & McKenna 1986) or 18 to 24 hours (Almond et al. 1998) later. The most widely used means of inseminating sows is the method developed by Melrose and O'Hagen (1959) and Rowson (1962), which involves passing a spiral rubber catheter into the cervix of the sow (Fig. 43.9). The catheter is rotated into the cervix until its spiral groove becomes locked into the cervical canal. The cervical lock prevents the catheter from becoming dislodged if the sow moves during insemination and prevents loss of the large volume of fluid that has to be inseminated.

However, there are very substantial losses of spermatozoa after insemination (reviewed by Roca et al. 2006a). Some 30% to 40% of the semen flows backwards out of the cervix, and of the spermatozoa that remain, many become trapped in the cervix or are eliminated by the immune system of the uterus. Hence, 90% of the spermatozoa are eliminated within 2 to 3 hours of insemination, and of the approximately 2.3×10^9 spermatozoa that are inseminated, only 1×10^5 become established at the uterotubal junction. This loss of spermatozoa is exacerbated when they are functionally impaired (e.g., when they have suffered damage through cryopreservation), with numbers at the uterotubal junction being tenfold lower than after insemination with liquid semen.



• **Fig. 43.9** Insemination of the sow, using a Melrose-type catheter.

In order to make the process of pig AI more efficient, two new approaches are being developed: more precise synchronisation of the timing of insemination with that of ovulations and deep intrauterine insemination.

Fertility is maximal when insemination is performed up to 12 to 18 hours before, or just after, ovulation (Nissen et al. 1997). Hence, if the timing of ovulation can be identified more accurately using signs other than those of behavioural oestrus, acceptable fertility might be achieved with a single insemination. Ultrasonography has been used, but scanning has to be undertaken frequently to give a useful prediction of the timing of ovulation (Bortolozzo et al. 2005, Sumransap et al. 2007). Hence ultrasound-based prediction of ovulation has yet to be refined to a point at which it is useful in commercial practice (Soede et al. 1998, Serret et al. 2005). Controlling the time of ovulation with gonadotrophins (GnRH, hCG, eCG) (de Rensis et al. 2003, Baer & Bilkei 2004, Kauffold et al. 2007), whether applied after the onset of observed oestrus or given at a fixed point after weaning, has been successfully used to allow adequate fertility to single inseminations. However, when single inseminations are used, the timing in relation to ovulation is critical (Garcia et al. 2007).

Intrauterine insemination has been performed in the caudal part of the uterus (postcervical insemination: PCI or deep within the uterine horns (deep uterine insemination; DUI)). Both are achieved by passing an insemination device through a conventional AI catheter; the device for PCI is rigid; that for DUI is longer, finer, and more flexible. PCI insemination requires more than 1×10^9 spermatozoa to obtain an acceptable litter size, although pregnancy rates are good even with lower numbers of sperm. In insemination trials with 1.5×10^9 spermatozoa in two doses, a farrowing rate of 84% with a litter size of 13.9 piglets was achieved (Hernández-Caravaca et al. 2012). However, success rates vary considerably with small numbers of spermatozoa (i.e., $0.5–1 \times 10^9$; Knox 2016). DUI with 600×10^6 spermatozoa is expected to give similar results in terms of pregnancy rate and litter size to conventional intracervical insemination with $2–3 \times 10^9$ spermatozoa (Roca et al. 2006b). Over 80% pregnancy rate has been achieved with 1×10^9 thawed spermatozoa deposited by DUI (Bathgate et al. 2008). Laparoscopic methods have been developed in which small numbers of spermatozoa are deposited into the ampullary part of the uterine tube very close to the time of ovulation (Vazquez et al. 2006). This has been used experimentally for sexed-semen inseminations, with good results (Roca et al. 2006b). However, the methods do not yet appear to be feasible for commercial use because they require general anaesthesia of the sow.

Insemination by Farm Staff

A semen delivery service was introduced into the UK in the mid-1960s (Melrose et al. 1968), and since then it has undergone steady expansion. Processed semen is dispatched from the processing centre to farms, where the farm staff, using conventional intracervical insemination catheters, inseminates the sows. This system allows double inseminations to be performed, and the risk of disease transmission is minimal. It requires some technical proficiency on the part of the farm staff in terms of oestrus detection as well as in the insemination procedure itself.

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The use of AI with delivered semen has also received considerable impetus from increased use of batch management of pig herds (see also Chapter 32). Thus, large groups of sows are weaned simultaneously in the knowledge that most of them will be in oestrus at a predictable interval after weaning. Standing or advance orders are placed for the semen to inseminate these sows, with small numbers of boars kept to mate the small proportion of sows that return to oestrus outside the predicted time period and to detect and mate animals that fail to conceive.

On-farm collection and insemination programmes can be used when large numbers of sows have to be inseminated as cheaply as possible. In this situation, inseminations are performed with raw or diluted semen immediately after collection. In a number of countries, AI centres have provided producers with semen extender for on-farm insemination programmes. This method minimises the risk of disease transmission but limits each farm to using the boars it has available. Access to performance/progeny-tested sires is therefore limited.

Inseminator Service

Technicians employed by AI centres transport the semen from the centre to the farm, where they inseminate the sows. This was the main form of insemination service in Western Europe when pig AI first started, but it has gradually lost favour, except in regions of exceptionally high pig density. Inseminator services generally provide only a single insemination of the sow in a given oestrus period, resulting in significantly lower conception rates and lower numbers of piglets per litter than are achieved by natural service (Reed 1982). Also, the cost of a technician service is high in relation to the value of the progeny, especially as litter sizes are lower than with natural service. Furthermore, itinerant inseminators are potential vectors for disease transmission between farms. Therefore the trends towards closed herds and 'minimal disease' precautions on most pig farms have mitigated against the use of such technicians.

Control of Infectious Diseases

A large number of pathogens can be transmitted through boar semen. The diseases that are specifically controlled by OIE are listed in Table 43.4.

Bacterial pathogens include *Brucella suis, Staphylococcus aureus, Streptococcus* spp., and *Mycoplasma* spp., which are transmitted by the venereal route and have significant effects upon sow fertility. *Leptospira* serovars and *Erysipelothrix rhusiopathiae,* although not directly spread through semen, are spread as urinary contaminants, so contamination of the semen through contact with urine or the presence of the organisms within the prepuce is likely (Cutler 1986).

More seriously, classical swine fever (hog cholera), African swine fever, and Aujeszky's disease can also be spread through semen, as can foot-and-mouth virus (Cutler 1986, Mengeling 1986, Almond et al. 1998). Porcine reproductive and respiratory syndrome (PPRS) virus can be shed in the semen and can be transmitted by insemination, although it is not specifically controlled by OIE (Yaeger et al. 1993). Likewise, parvovirus and Japanese encephalitis can also potentially be transmitted by semen (Thibier & Guerin 2000).

Routine serological testing of boars is undertaken by AI centres to ensure that they do not carry either venereally transmitted diseases or other conditions that might jeopardise the health status of the recipient herd. Antibiotics are added to semen to remove nonspecific and pathogenical bacteria. However, the most valuable means of controlling disease transmission is to quarantine incoming boars for a month, during which time observations for signs of clinical disease and/or further serological testing are undertaken.

Artificial Insemination of Horses

Artificial insemination of horses has been practised for many years. Initially, most of the breeding by AI took place in Eastern Europe, the former USSR (Tischner 1992), and China. Conversely, development of AI in horses in western countries was hampered for a long time by the refusal of key registration authorities to allow the registration of foals bred by AI. However, the recent acceptance of such registrations by the American Quarter Horse and American Paint Horse associations (Loomis 2001) has provided a significant impetus to the development of equine AI. Thus, although the Thoroughbred Breeders Association remains unwilling to allow registration of foals conceived by AI, most other breed societies allow restricted or unrestricted registrations of AI-bred foals. In most parts of Europe, up to 90% of Warmblood mares are now bred by AI.

There are a number of technical difficulties that have to be overcome in the development of AI programmes for horses:

- Semen collection: Coitus in horses is relatively protracted, which makes semen collection rather more difficult than in other large herbivores. In some countries, most of the seminal plasma is removed by centrifugation to enhance spermatozoa survival (Brinsko & Varner 1993).
- Storage of semen: Stallion semen is relatively susceptible to damage due to cold shock and cryopreservation (Amann 1984).
- Insemination: Synchronising insemination with ovulation has to be accurate. However, because of the variability and duration of oestrus in the mare, predicting the time of ovulation is difficult without regular and frequent examination. A commonly used practice is to inseminate every 48 hours once a dominant follicle has been detected until ovulation occurs. On the other hand, insemination of the mare is much easier than insemination of the cow because the equine cervix is soft and does not have a convoluted luminal canal.

Collection and Storage of Semen

Semen is normally collected by AV (see Chapter 35). Some people prefer to use an open-ended AV to allow collection of the separate fractions of the ejaculate. Alternatively, the gel fraction can be separated by use of a nylon mesh filter placed in the AV or semen bottle at the time of collection or by filtration through gauze after collection. The latter is associated with a greater loss of spermatozoa than other methods (Blanchard et al. 2003). In commercial AI programmes, it may be advantageous to train stallions to mount a 'breeding phantom' dummy-mounting device, in preference to the use of mares. Ground collection is also possible; this method is thought to place less strain on the back and hind legs than mounting a phantom.

Liquid Semen

After collection, equine semen requires careful temperature control to prevent damage to the spermatozoa by cold shock (Brinsko & Varner 1993). If the mares that are to be inseminated are close by, insemination can be performed directly using raw semen, but it is generally preferable to place the semen in an extender even if it is for direct insemination straight away. Simple extenders (Table 43.11), such as skimmed milk plus antibiotics used at a ratio of

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TABLE 43.11 Diluents for short-term storage of equine semen at 4°C

Component (g/L)	Skimmed Milk Diluent (Kenney)	Modified Kenney Extender	Egg Yolk–Glucose
Fat-free dried milk powder	24	24	
Glucose	49	26.5	70
Sucrose		40	
Sodium hydrogen carbonate	1.5		
Egg yolk			70 mL
Antibiotics	Penicillin G, streptomycin, gentamicin, polymyxin B, and/or amikacin are commonly added		

Derived from: Kenney et al. 1975, Tischner 1992, Brinsko & Varner 1993, Blanchard et al. 2003.

1:1 or 1:2, with semen maintained at ambient temperatures are adequate if the semen is to be used within 6 hours of collection (Samper 2007).

Equine semen can also be stored for longer periods of time before insemination if it is cooled. Most studies (see Batellier et al. 2001) have concluded that storage at 4° to 6°C is the ideal temperature (although there are some reports of better results at 15° or even 20°C). Extenders for cooled storage are mostly based upon milk, using either skimmed milk, cream-gel ('half-and-half' cream partially set with gelatin), or fat-free dried milk powder. Kenney's diluent (Kenney et al. 1975) and its very close derivative (Francl et al. 1987) EZ Mixin (Animal Reproduction Systems, Chino, CA) are probably the most widely used diluents in the United States because of their simplicity of use and the ease with which spermatozoa can be visualised after dilution (Blanchard et al. 2003) (Table 43.11).

Other extenders that are used for cooled storage of equine semen are based on milk plus egg yolk or are chemically defined (e.g., INRA-96; Batellier et al. 1998). Extenders based on egg yolk appear to provide best protection when seminal plasma is removed (Jasko et al. 1992), whereas milk-based diluents support cooled storage less well when seminal plasma is completely removed, the effect of which can be overcome if the extender is supplemented with salts such as Tyrode's (Rigby et al. 2001). However, milk-based diluents that are supplemented with Tyrode's salts require the removal of seminal plasma for maximal survival of spermatozoa (Katila et al. 2005). The defined diluent INRA-96, which is based on Hank's salts supplemented with 67 mmol/L glucose, 126 mmol/L lactose, and 27 g/L native phosphocaseinate (Batellier et al. 1998, Pagl et al. 2006), gives pregnancy rates that are better than simple skimmed milk diluents, especially for stallions whose semen does not respond well to simple extenders (Batellier et al. 2001). Webb and Humes (2006) showed that further improvements to the performance of semen in INRA-96 can be obtained by removal of the seminal plasma by centrifugation. However, although motility of spermatozoa may be improved by removal of most of the seminal plasma, there may be an increase in chromatin damage (Morrell et al. 2010). Masuda et al. (2004) suggested that an extender based on 2% milk casein plus 5% egg yolk also gave good results.

With these diluents, the rate of cooling from body temperature to 19°C is not critical, but between 19° and 8°C, slow cooling (ideally 0.1°–0.3°/min; no more than 0.5°C/minute) is necessary to avoid causing cold shock (Douglas-Hamilton et al. 1984, Katila 1997). Below 8°C, rate of cooling is not critical. Controlling the rate of cooling semen in the field has been greatly facilitated by cooled storage units such as the Equitainer (Hamilton Research, Inc., South Hamilton, MA). In this system, diluted semen is placed in an insulated container with prechilled cooling packs that reduce the temperature in the semen at an appropriate rate to prevent cold shock developing. Such devices can maintain semen fertility at acceptable levels for approximately 48 hours. Air should be excluded from storage vessels as the survival of spermatozoa is significantly greater when no air is present (Katila 1997, Batellier et al. 2001) because of the effects of aerobic respiration and lipid peroxidation. Addition of antioxidants, such as ascorbic acid, pyruvate, and, most recently, N-acetyl cysteine (Pagl et al. 2006), is therefore advocated as a means of increasing the longevity of spermatozoa in cooled storage.

Cryopreservation

Stallion semen has been successfully cryopreserved (Pickett & Amann 1993) using low concentrations of glycerol in diluents containing sugars, egg yolk, and/or skimmed milk. Before freezing, most of the seminal plasma is removed by centrifugation, usually after temporary extension of the semen in a diluent that will maintain its viability at ambient temperatures. Various diluents have been used for this step, including UHT skimmed milk, Kenney's diluent, lactose-EDTA, INRA-82 (glucose, lactose, rafinose, citrate, Hepes, plus egg yolk (Vidament 2005)), and EDTA–citrate. To minimise damage to spermatozoa during the centrifugation process, semen may be 'cushioned', by layering it on to a high viscosity solution before centrifugation. High concentrations of glucose or dense, inert, isotonic substances (e.g., Cushion-Fluid, Minitube; Landshut, Germany) (Sieme et al. 2006) have been used as the basis for cushioning media for centrifugation. After discarding the supernatant, the semen is resuspended in the freezing extender. It is then slowly cooled from ambient temperatures to 4°C because slow cooling over this phase is preferable to rapid cooling.

Freezing extenders are typically based on sugars, buffers, and egg yolk; many are available commercially and many others have been described in the literature. Among these, Kenney's plus 4% clarified egg yolk, INRA-82 plus 20% egg yolk, or lactose–EDTA plus 20% egg yolk (Pickett & Amman 1993, Loomis 2006) have been widely used as freezing media. Subsequent studies have suggested that defined media containing egg- or soy-derived phosphatidylcholine (Ricker et al. 2006) maintain spermatozoal survival during freezing at least as well as extenders containing whole egg yolk. Glycerol is the best tolerated cryoprotectant and is typically used at a final concentration of 2.5%, although some diluents use concentrations of up to 4% (Cochran et al. 1984, Heitland et al. 1996). More recently, there has been a move towards substituting methyl formamide and other amides for some of the glycerol in cryoextenders for stallion semen (Alvarenga et al. 2005). This substitution seems to aid cryosurvival for some individuals that display poor cryosurvival when only glycerol is used.

The use of 0.5 mL paillettes has superseded older, larger volume containers for freezing equine semen: little semen is now frozen in other containers. The insemination dose is commonly loaded into four to eight straws (Samper 2007). Freezing may be over the vapour of liquid nitrogen or as pellets on the surface of blocks of solid carbon dioxide. When programmable freezers are used,

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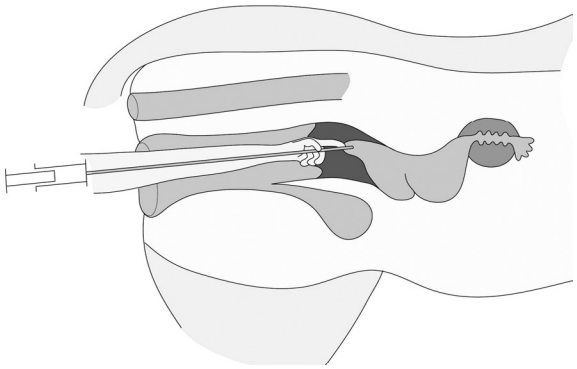
cooling rates of 40°C/min to −60°C then 60°C/min until −140°C is reached (thereafter plunged into liquid nitrogen) have given good results (Vidament 2005).

Thawing rates are more critical for cryopreserved equine sperm than for many other species. Loomis and Squires (2005) suggest that semen frozen in 0.5 mL straws should be thawed at 37°C for a minimum of 20 seconds. It is important that the temperature of the thawing water is accurately controlled because spermatozoa die rapidly if the temperature is raised to 39°C or 40°C. Higher thawing temperatures (e.g., 75°C for 7 seconds) give better post-thaw results, provided the straws are removed at the correct time; however, it is difficult to ensure that this occurs in field use. The contents of four to eight straws are pooled before insemination. When semen is supplied in 4 to 5 mL tubes, thawing should be at 50°C for 45 seconds.

Evaluating the fertility of cryopreserved equine semen is not a straightforward process. Post-thaw motility is a poor predictor of fertility in all species; it identifies the worst ejaculates but fails to discriminate between levels of fertility. In the stallion, it is particularly unreliable (Pickett & Amann 1993) given the minimal amount of reliable data on conception rates in mares. However, attempts have been made to find objective measurements that have high levels of correlation with fertility. Christensen et al. (1996) used *in vitro* induction of acrosome reactions as a predictor of fertility, whilst Samper (1992) and Hellander (1992) have used combinations of glass–wool/Sephadex filtration and motility estimates to predict fertility. Nevertheless, despite the progress that these methods represent, it remains difficult to predict the fertility of a stallion's semen after cryopreservation.

Insemination

The standard method of insemination with cooled or frozen semen is to deposit it into the caudal part of the uterine body via a catheter passed through the cervix (Fig. 43.10). Insemination is best performed with the mare restrained in stocks. After applying a tail bandage and cleaning the perineal area, a hand is inserted into the vagina and the cervix is located. The index finger is inserted into the cervix, and an insemination catheter is passed through the vagina, then alongside the index finger, and so into the uterus. A vaginal speculum can be used to aid locating the cervix. Semen is deposited slowly, in volumes ranging from 0.5 to 80 mL. Most inseminations use a low volume of fluid as there is little benefit from higher volumes upon uterine contractility, and there is a greater risk of retrograde flow from the cervix with larger volumes.



• **Fig. 43.10** Insemination of the mare.

Timing of Insemination

Insemination with cooled semen should take place somewhere between 48 hours before and approximately 6 hours after ovulation. Insemination later than 6 hours after ovulation may result in conception but is more likely to result in embryonic death. The optimal timing depends, however, upon the success with which the spermatozoa have survived the cooling process: the semen of some stallions needs to be inseminated within a few hours of ovulation if it is to achieve an acceptable result. Samper (2007) suggests that a fertile mare can be inseminated once or twice (12–24 hours apart) with good quality semen, but if the semen is of poorer quality or the fertility of the mare questionable, a single insemination as close as possible to the time of ovulation is preferable.

Insemination with frozen–thawed semen needs to be performed between 12 hours before and 6 hours after ovulation. Regular examination of the mare is required to ensure that this timing is achieved. In practice, most mares are inseminated once within 6 hours after ovulation or twice, once before and once after ovulation (Loomis & Squires 2005). Mares are often given human chorionic gonadotrophin (hCG) or a gonadotrophin-releasing hormone (GnRH) analogue to induce ovulation and thus control the timing of insemination more precisely. This has the added advantage of reducing the number of occasions on which the mare has to be examined. Samper (2001) suggested giving 2500 IU hCG once a follicle larger than 35 mm is present, then examining the mare's ovaries every 6 to 8 hours to pinpoint the time of ovulation, which will occur 36 ± 17 hours after treatment. The GnRH analogue deslorelin (Ovuplant) can be given as a short-term implant, resulting in a much closer regulation of the time of ovulation (Hemberg et al. 2006), with ovulation occurring at 38 ± 2 hours post-treatment, so that it is only necessary to examine mares at 36 and 41 hours post-implantation to determine the point of ovulation.

Spermatozoa Numbers and Conception Rates

Cooled Semen

Cooled semen inseminations are typically made with 250 to 500 × 10^9 progressively motile spermatozoa (Brinsko 2006), commonly doubled to 1 × 10^9 motile sperm when insemination is to be delayed by more than 24 hours. Conception rates of 73% to 75% have been recorded for this method. However, this number of spermatozoa almost certainly represents a wastage, at least in fertile stallions, as 100 × 10^6 motile spermatozoa have been used for insemination without any loss of fertility (Pace & Sullivan 1975, Pickett 1980), although lower numbers (e.g., 50 × 10^6) give more variable results (Householder et al. 1981). On the other hand, the use of higher numbers of spermatozoa is said to eliminate much of the variation between stallions.

There is, however, a great deal of variability in the quality of cooled semen that is supplied to horse breeders. Metcalf (1998) showed that semen rated as excellent or good on the basis of percentage of motile and morphologically normal sperm had higher (87% and 62%, respectively) pregnancy rates than semen rated as fair or poor (33% and 11%). Loomis (2001) reported a wide range of numbers of progressively motile sperm in cooled semen (mean: 598 × 10^6; SD: 604 × 10^6; range: 8–4257 × 10^6). Moreover, although controlled trials have shown that it is possible to reduce sperm numbers without loss of fertility, Brinsko (2006) showed evidence from use in the field that doses of less than 500 × 10^6 sperm are associated with much lower pregnancy rates (20%) than doses of more than 500 × 10^6 spermatozoa.

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Frozen Semen

Frozen semen inseminations typically contain between 150 and 600×10^6 motile sperm. With most stallions having post-thaw recovery rates of 40% to 70% (Vidament 2005), this necessitates a total insemination dose of around 800×10^6 spermatozoa. The fertility of frozen semen in commercial programmes has been reported to range between 32% and 73% per cycle and 56% to 89% over the breeding season (Loomis 2001). Various combinations of spermatozoal numbers and insemination timings have been reported: Vidament (2005) suggested that best results are achieved with up to two daily inseminations with 300 or 400×10^6 spermatozoa from the time of hCG administration or a single postovulation insemination, figures with which Newcombe et al. (2005) concurred.

New Insemination Methods for Compromised Spermatozoa

Losses of spermatozoa in traversing the uterus from the site of insemination to the uterotubal junction are high, especially when the functional integrity of the spermatozoa has been compromised by freezing or by sex sorting. Deep intrauterine insemination has been investigated as a means of reducing the numbers of spermatozoa and improving the pregnancy rates. In this method, a semirigid or flexible insemination catheter is guided to the cranial part of the uterine horn, and 20 to 200×10^6 spermatozoa are deposited. The catheter can be guided per rectum or using ultrasonography (Sieme et al. 2003). Early attempts at this method resulted in high embryonic loss rates due to traumatic injury to the uterus by the catheter (Morris 2006a), although this problem now appears to have been resolved (Sanchez et al. 2005). Whether an improvement in conception rates is achieved over conventional methods remains unclear: neither Squires et al. (2003) nor Sanchez et al. (2005) saw improved pregnancy rates, although sperm numbers to achieve conceptions were successfully reduced. A reduction in the incidence of postbreeding endometritis may be obtained if fewer spermatozoa are inseminated (Samper & Plough 2010).

Direct insemination on to the uterotubal junction has been investigated as an alternative to deep intrauterine insemination. Hysteroscopic insemination at the uterotubal junction allows reduction of sperm numbers to 10 to 25×10^6 (Sieme et al. 2004, Ball 2006) or even as low as 1 to 5×10^6 (Morris et al. 2003, Sieme et al. 2003, Morris 2006b), which allows acceptable pregnancy rates to be achieved even with sex-sorted sperm (Lindsey et al. 2001). Finally, direct insemination into the uterine tube has also been successfully used for insemination of compromised sperm (Lindsey et al. 2001, Morris 2004). Although these techniques have significant benefits in terms of improved usage of spermatozoa, especially when only small numbers are available (e.g., with sexed spermatozoa), the costs are substantial and training is required. Thus they are not in widespread use in the field.

Control of Infectious Diseases

A considerable number of pathogens are transmissible through equine semen (Table 43.1). Most importantly, this includes equine viral arteritis, which can be shed in the semen of stallions for long periods of time after infection. Because of the difficulties of identifying shedding stallions, the use of methods for processing semen that remove the virus has been recommended (Morrell & Geraghty 2006, Morrell et al. 2014). Many other viruses can be transmitted through semen, including EHV-III, equine infectious anaemia, and, possibly, EHV-II and vesicular stomatitis. The case with EHV-I is less clear-cut for, although its transmission through semen appears likely, no recorded cases have occurred (Klug & Sieme 1992). Many nonspecific bacterial contaminants of semen may cause infertility in inseminated mares. The presence of β-haemolytic streptococci in semen is associated with reduced fertility of mares (Klug & Sieme 1992), as are haemolytic *E. coli, Staphylococcus aureus,* and *Pseudomonas aeruginosa. Klebsiella* spp. are of more clear-cut significance as a cause of infertility, whereas *Taylorella equigenitalis,* the causal organism of contagious equine metritis, is an important venereal pathogen, although not controlled by OIE. Within its geographical area of distribution, *Trypanosoma equiperdum,* the protozoon responsible for dourine, is venereally transmitted. Legislative control exists in many countries to control the spread of equine viral arteritis, equine infectious anaemia, contagious equine metritis, and dourine, at least in the bloodstock industries, but control of venereal diseases in ponies and riding horses is often minimal. The simple precautions of serological examination of stallions for the presence of viral infection and serial bacteriological examination of their external genitalia before using the animals as AI (or, indeed, natural service) sires are all that is required to control most venereal pathogens, yet, all too often, this does not occur until after the onset of a disease outbreak. However, a negative serology result only indicates the absence of an antibody response to the virus, not the absence of infection.

Artificial Insemination of Dogs

It was upon the dog that the earliest recorded studies of AI were undertaken, by the Italian natural philosopher Spallanzani. Despite such an impeccable pedigree, demand for AI of dogs has been slow to develop, as there has historically been a significant degree of resistance to it by many breed societies. This position has changed in more recent years (UK Kennel Club 2017), so the use of AI in dogs has increased markedly. In parallel, methods have been developed for breeding nondomestic Canidae for conservation or commercial purposes.

Collection and Storage of Semen

Semen is usually collected from the dog by digital manipulation (see also Chapter 35). The use of electroejaculation under general anaesthesia has also been attempted (Kutzler 2005). The preejaculatory fluid, sperm-rich fraction and a little of the postejaculatory (prostatic) fluid are collected. The whole ejaculate may be immediately inseminated into the bitch's vagina, but it is more common to dilute the semen so that multiple inseminations can be performed. Where the whole ejaculate is used, only a few millilitres of prostatic fluid should be collected.

Liquid Semen

Dog semen can be stored in a cooled condition for 24 to 72 hours at 4°C, after dilution in simple diluents (e.g., skimmed milk) (Harrop 1962). This is generally long enough for at least two inseminations to be performed on the bitch, or for air freight transport to most international destinations. Hence, this method is very widely used in dog AI (Pena et al. 2006). Better longevity of semen can be achieved with the use of diluents such as Kenney's diluent for equine semen (Bouchard et al. 1990; Table 43.11) or Tris–glucose–egg yolk (Iguer-Ouada & Verstegen 2001; Table 43.12). Removal of the prostatic fluid by centrifugation improves

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TABLE 43.12 Diluents for cooled storage of canine semen

Component	Tris–Glucose/ Tris–Fructose	Skimmed Milk
Tris (g/L)	30.25	
Sodium citrate monohydrate (g/L)	17	
Glucose or fructose (g/L)	12.5	
Skimmed milk		80%
Buffer	80%	
Egg yolk	20%	20%
Antibiotics	Commonly penicillin, streptomycin	

Derived from: Iguer-Ouada & Verstegen 2001, Romagnoli 2002.

storage and viability, especially when samples are dilute (Romagnoli 2002), although semen can be preserved successfully without its removal (England & Ponzio 1996). Cooling from body temperature to 4°C should be slow to prevent cold shock: Bouchard et al. (1990) recommended cooling at 0.3° to 1.0°C/min. This rate can be achieved in practice by placing the diluted semen in a container filled with water at room temperature, which is then placed at 4°C overnight (Pena et al. 2006). The use of the Equitainer as a means of cooling/transporting canine semen has also been recommended. Dilution rates have to take into account the volume that can be inseminated into the bitch as well as the density of the semen but should aim to achieve at least 150×10^6 progressive motile sperm.

Cryopreservation

Cryopreservation of dog semen is more difficult than for many species, but success has been improving over recent years. Many different diluents have been tried, largely on an empirical basis or because they work for other species. Egg yolk appears to be an important component of most diluents. Likewise, many different glycerolisation regimens have been employed in an attempt to achieve a satisfactory compromise between its toxic effects upon sperm and the concentrations needed to give effective cryoprotection (England 1993). Although the optimal glycerol concentration appears to be about 6%, its addition in one or multiple steps does not have a major effect upon post-thaw survival (Silva et al. 2003).

Consequently, there are many regimens for freezing semen. Two methods are described as examples of those in current use. Thomassen et al. (2006) diluted semen in a Tris–fructose–citric acid extender, with 8% glycerol and 20% egg yolk, in a single step at 35°C. It was then cooled slowly to 5°C before packaging into 0.5 mL paillettes for freezing. Pena et al (2006) diluted semen 1:1 in a Tris–glucose extender before centrifugation to separate the seminal plasma. After that the spermatozoa were resuspended in further Tris–glucose–egg yolk diluent, with a two-step glycerolisation (3% at room temperature, followed by slow cooling to 5°C, then diluted further with a similar diluent with a higher content of glycerol, to provide a final glycerol concentration of 7%). Freezing is also in 0.5 mL paillettes.

Early methods for freezing relied on freezing in pellets on blocks of solid carbon dioxide or in paillettes suspended in the vapour of liquid nitrogen. The results achieved by these methods are remarkably similar: in 2001, Nianski et al. found no difference between the post-thaw recovery of semen frozen as pellets or in 0.25 or 0.5 mL paillettes. The use of programmable freezers has the potential to result in significant improvements in post-thaw recovery rates, although, as the optimal cooling rate is determined by the concentration of glycerol and the other components of the diluent, it is difficult to generalise about optimal cooling rates. Thus, Rota et al. (1998) found no difference in post-thaw recovery between cooling at 10°C/min and 50°C/min between −10°C and −40°C; however, a cooling rate of 30°C/min is commonly recommended over this period. Thawing at 70°C in water for 8 seconds provides good post-thaw recoveries, although 37°C for 30 seconds is also used (Thomassen et al. 2006).

Insemination

Liquid Semen

When the reason for AI is failure of copulation, semen is collected from the dog by digital manipulation, and the whole ejaculate may be inseminated into the vagina of the bitch immediately after collection. It may, however, be preferable to extend the semen, inseminating one portion immediately and the remainder 48 hours later.

Similarly, when cooled semen has been transported from the sire to the bitch, insemination may be performed upon receipt of the semen and repeated 48 hours later. It is therefore imperative to ensure that the recipient bitch is approaching the fertile period of oestrus before semen is collected.

When fresh or cooled semen is used, intravaginal insemination is undertaken, with the semen deposited as close as possible to the external os of the cervix (Burke 1986). The semen may be deposited through a shortened bovine insemination catheter, which may require the use of a speculum to be guided into the correct site. Some authors recommend inserting one or two fingers into the vulva after insemination, in order to promote the motility of the female genital tract that normally occurs during the copulatory tie. Alternatively, an Osiris catheter, which consists of an outer sheath with a Foley bulb and an inner catheter, can be used (Nianski 2006). The inflatable balloon is considered to both simulate the copulatory tie and prevent retrograde flow of semen. However, there is no unequivocal evidence that this type of catheter improves conception rates beyond those achieved with simple catheters. Whichever method is used, the hindquarters of the bitch should be raised for a few minutes after insemination to prevent retrograde loss of semen. Pregnancy rates of approximately 80% can be expected with fresh semen (England & Miller 2008).

Frozen Semen

Conception rates using frozen semen are poorer with intravaginal than intrauterine insemination. Hence direct intrauterine insemination, either via the cervix or directly via surgery or laparoscopy, is currently recommended. Linde-Forsberg et al. (1999) reported whelping rates of nearly 85% with intrauterine insemination compared with approximately 60% with the intravaginal route. However, higher pregnancy rates were achieved when multiple intravaginal inseminations were performed, although there was no benefit from multiple inseminations by the intrauterine route. The Osiris catheter has been used to try to improve conception rates to intravaginal insemination (Nianski 2006); however, whelping rates by this method were also approximately 60%.

Passing a catheter through the cervix is difficult because of the length of the vagina, the length of the cervix, and the site of its external os (England & Lofstedt 2000). However, the Norwegian

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catheter, which consists of a 2 mm catheter inside a Teflon sheath, has been used successfully, although considerable practice is required to master the technique (Romagnoli 2002). Thomassen et al. (2006) describe the method of intrauterine insemination as follows: Insemination is performed on standing bitches, which normally do not require sedation. The cervix is fixed through the abdominal wall with one hand as the steel catheter is manipulated through the cervix into the uterus with the other. After deposition of the semen, care is taken to avoid backflow by ensuring that the plastic sheath remains in close contact with the catheter and by elevating the hindquarters of the bitch (as described previously). As an alternative to transabdominal fixation of the cervix, an endoscope can be used to locate the cervical os. Insemination is easier in bitches of moderate size than in giant and miniature breeds, and is more difficult in animals that are excessively fat. However, pregnancy rates are reported to be similar to the Scandinavian method described previously (Payan-Carreira et al. 2011).

Insemination via a surgical laparotomy or via laparoscopy can also be used for insemination of frozen semen, especially when practitioners are not confident with the transcervical method.

Timing of Insemination

The bitch has a prolonged period of receptivity to the male but a relatively short fertilisation period (see Figs. 33.13–33.15). Thus the fertility achieved in canine AI primarily depends on achieving correct timing of insemination in relation to that of ovulation. When natural service is used, many breeders allow bitches only a single mating, which typically occurs 12 days after the onset of prooestral bleeding. For successful AI, much closer attention to the time of the fertilisation period is needed, especially when cryopreserved semen is used, although more latitude exists when cooled semen is used (Jeffcoate & Lindsay 1989, Linde-Forsberg & Forsberg 1989, 1993, Morton & Bruce 1989). The inherent fertility of the dog is also of importance, with the longevity of his spermatozoa in the female tract being a critical determinant of fertility. Dogs with long-lived spermatozoa can achieve pregnancies even if the timing of insemination is not optimal, whereas with dogs whose sperm have poor survival, sperm death is more likely to have occurred before ovulation under such circumstances (see Figs. 33.13–33.15).

The timing of ovulation can be predicted from the preovulatory rise in circulating progesterone concentrations or measurement of LH concentrations. Alternatively, the ovaries can be examined by ultrasonography. Vaginal cytology, although useful as an indicator of the approach of ovulation, is too imprecise for frozen semen inseminations. Measuring serum progesterone concentrations daily after the onset of oestrus (or after the appearance of a high percentage of cornified vaginal epithelial cells) provides a reasonably good predictor of the timing of ovulation. Progesterone concentrations rise from baseline values to 2.0 to 3.0 ng/mL on the day of the LH surge, thereafter increasing to 4.0 to 8.0 ng/mL on the day of ovulation, and 10 to 25 ng/mL during the 2 days after ovulation (Romagnoli 2002). Hence the first rise in progesterone concentrations can be taken as an indicator of the LH surge. Alternatively, LH can be measured directly using an 'animal-side' assay method (e.g., the semiquantitative Witness-LH, Zoetis, Parsippany, NJ); the assay is intended for use with serum, although it has also been used to detect LH in urine (Durrant et al. 2006). Insemination should ideally be performed 2 to 3 days after ovulation. Thomassen et al. (2006) showed that insemination at the optimal time results in a higher whelping rate and litter size (78% and 5.8 ± 0.2 pups, respectively) than late insemination (56% and 4.5 ± 0.5 pups). Another suggestion for fresh or cooled semen is to inseminate on the day of ovulation and again 2 days later, whereas with frozen semen, the first insemination is done 2 days after ovulation with the second 48 hours later (Payan-Carreira et al. 2011).

Artificial Insemination of Cats

Insemination of domestic cats is undertaken relatively rarely because of the small volumes of semen obtained (0.05–0.25 mL) and the need for anaesthesia for collection and insemination. However, it is used in conserving rare breeds or for international trade and especially for the conservation of wild Felidae, many of these species being endangered. Recent reviews of feline AI have been published by Rijsselaere and van Soom (2010) and England and Russo (2014).

Semen is collected by electroejaculation (Zambelli & Cunto 2006) under general anaesthesia or by urethral catherisation under sedation with medetomidine, which stimulates the α_2-adrenergic receptors (Rijsselaere & van Soom 2010). Semen can also be harvested postcastration from the epididymides, vasa deferentia, or even from minced testis tissue.

Cooled storage of semen requires prevention of cold shock. Egg yolk is less effective at protecting cat semen against cold shock than it is in most other domestic species and, although simple Test (Tes+Tris)-yolk will maintain sperm viability at 5°C, the low density lipoprotein fraction is far more effective than whole yolk (Glover & Watson 1987). Such diluents can maintain semen quality for approximately 24 hours, with occasional reports of longer periods of storage.

Similar extenders are used for freezing as those for cooled storage or with extenders based on Tris, citrate, glucose, fructose, or lactose plus egg yolk. Glycerol is the best cryoprotectant for cat semen, but because of sensitivity to its toxic effects, it is usually added at concentrations of up to 4%. Antioxidants, such as taurine, appear important in the maintenance of post-thaw viability. After loading into paillettes, semen can be frozen in liquid nitrogen vapour, in programmable freezers (at a cooling rate of 10°C/min between +5° and −80°C) or, less successfully, in pellets on dry ice.

Ovulation has to be induced before insemination. The most commonly used hormone for this purpose is hCG: one or two doses are given and insemination performed 15 to 30 hours later. Opinions vary concerning whether it is better to inseminate before or after ovulation has occurred because, with natural mating, ovulation would occur approximately 24 to 48 hours after coitus (England & Russo 2014). Intravaginal insemination has been reported, with pregnancy rates varying from 11% to 78% (reviewed by Rijsselaere & van Soom 2010). In domestic cats, intrauterine insemination via a laparotomy is more convenient, yielding pregnancy rates of 80% for fresh semen and 0% to 20% for frozen semen. However, in some countries this procedure is considered to be invasive and unethical and is prohibited for normal breeding. Sperm doses are 50 to 80 $\times 10^6$ for vaginal insemination and 20 to 50 $\times 10^6$ for intrauterine insemination (England & Russo 2014).

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Embryo Transfer and Other Assisted Reproductive Technologies

HENRIK CALLESEN, INGRID BRÜCK BØGH AND TORBEN GREVE

Introduction

In animal husbandry, breeding and selection for special phenotypic characteristics or superior performance has been conducted by humans for thousands of years, but it was not until the last century that the first systematic assisted reproductive technology (ART) was developed and soon introduced in practical breeding. Initially, artificial insemination was the only one, but subsequently, a number of other ARTs have followed. Several of these ARTs, including artificial insemination, embryo transfer, and *in vitro* embryo production (IVP), were initially developed in farm animals for genetic progress or in research to establish animal models, but they have increasingly been modified and subsequently used in companion animals. Some of these techniques, such as intracytoplasmic sperm injection (ICSI), oocyte recovery, and gamete intrafallopian tube transfer (GIFT), were primarily developed in humans as part of a therapy for sub- or infertility. Subsequently, they have been adapted to different animal species either to circumvent specific causes of sub- or infertility or to facilitate basic and applied research. Within the context of this book, the present chapter will describe the basic principles of different ARTs, with emphasis on the large domestic animal species and with focus on the clinical aspects that are relevant to the veterinary practitioner, including obstetrics, pathology, and biosecurity.

Definition

Reproduction is basically the physiological process which guarantees the preservation of the species, and in nature, it requires mating. However, this physical encounter can be circumvented by different technical means that involve human intervention. ARTs that may have relevance for the veterinarian comprise: artificial insemination; embryo transfer, including superovulation and oestrous synchronisation; oocyte recovery for *in vitro* production; and manipulation of embryos, including production of embryos by means of somatic cell nuclear transfer (cloning), transgenic embryos as well as cryopreservation of oocytes and embryos.

Milestones in the Development of Assisted Reproductive Technologies

In 1890 Heape carried out the first successful embryo transfer in rabbits (Heape 1890) and the first calf after embryo transfer was born in 1951 (Willett et al. 1953). Research and development in ARTs have subsequently been substantial, especially in cattle. In the 1960s a technique for nonsurgical embryo transfer was introduced (Sugie 1965), and higher embryo yields from selected individuals were obtained by hormonal induction of multiple ovulations (superovulation). During the last 40 years hormonal regimens for superovulation in cattle have been refined and combined with subsequent transfer of fresh or cryopreserved embryos, a combination of techniques called multiple ovulation and embryo transfer (MOET). Today, this technique has become a routine procedure in many countries, and in 2015 more than 520,000 *in vivo* derived bovine embryos were transferred to recipients worldwide, mainly in North America and Europe (Perry 2016).

In the horse, increasing interest in maximising the reproductive potential of genetically valuable animals led to the first successful nonsurgical transfer of an equine embryo in the early 1970s (Oguri & Tsutsumi 1974, Allen & Rowson 1975, Vogelsand et al. 1947). Since then, the techniques have been improved so acceptable pregnancy rates can be achieved, and in 2015 at least 21,000 equine embryos were transferred to recipients (Perry 2016). Nevertheless, the two major limiting factors are still the lack of an efficient method for superovulation of mares and barriers with cryopreservation of horse embryos.

The first lambs were born after embryo transfer in 1949 (Warwick & Berry 1949). In sheep and goats, embryo transfer is used primarily to enable the importation of new breeds; however, the application is limited because of the high cost and low efficiency of the technology (Warwick & Berry 1949). At least 31,000 sheep and 11,000 goat embryos were transferred in 2015 (Perry 2016).

In the pig the use of embryo transfer for commercial breeding purposes has been and still is limited to a few hundreds (Perry 2016). On the other hand, the technology is widely used for research, but no systematic collection of data is available. In 2015, it can be roughly estimated that more than 15,000 porcine embryos have been transferred for research purposes, especially in relation to somatic cell nuclear transfer (cloning) and transgenesis. The majority of these embryos have been transferred fresh, even though vitrification of porcine embryos has become a possibility (Sanchez-Osorio et al. 2010).

In vitro embryo production has been developed in several species. The first child conceived by *in vitro* fertilisation (IVF) was born in 1978 (Steptoe & Edwards 1978), followed in 1981 by the first calf (Brackett et al. 1982). Since then the techniques for *in vitro*

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• **Fig. 44.1** Fifteen calves produced by nonsurgical embryo transfer after superovulation from one single donor. (Courtesy Søren Ernst Madsen, Trans Embryo, Denmark.)

production of bovine embryos improved rapidly, and soon it was possible to produce calves from oocytes, both matured and fertilised *in vitro* (Lu et al. 1988; Xu et al. 1988). Today, the complete *in vitro* technique with maturation, fertilisation, and culture has found a wide application both as a research tool and in veterinary practice (Blondin 2017). In 2015 the number of transfers of *in vitro* produced bovine embryos to recipients worldwide exceeded 400,000, mostly in South and North America (Perry 2016).

In 1991, two foals conceived by *in vitro* fertilisation of *in vivo* matured oocytes were born in France (Palmer et al. 1991, Bézard et al. 1992), but in spite of many attempts worldwide, this success has never been repeated. Instead, ICSI is a more reliable method for the *in vitro* production of equine embryos, and the first ICSI foal was born in 1998 after surgical transfer into the oviduct (Cochran et al. 1998). Subsequently, *in vitro* culture of the early embryo to the blastocyst stage has been developed, allowing for nonsurgical transfer (Carnevale et al. 2004). Since then the technique has been optimised and has found clinical use in cases in which natural breeding of genetically valuable mares or stallions is not possible, even allowing for foals being bred from mares immediately after their death (Carnevale et al. 2004, Hinrichs et al. 2012). The total number of ICSI-produced embryos transferred worldwide in 2015 is estimated to be fewer than 5000 (Hinrichs 2016).

The birth of the first mice and calf after cryopreservation was reported in the early 1970s (Whittingham et al. 1972, Wilmut & Rowson 1973), and this led to an entirely new situation for ARTs in domestic animals. Cryopreservation made it possible to store embryos for many years and transfer them independently of the date and site of recovery. This has been extremely important both for research purposes in rodents and many other species, but also for commercial embryo transfer in the domestic animal species.

Willadsen's original studies on embryo cloning in sheep (Willadsen 1986) initiated another important area for both research and commercial use of ARTs, and the birth of the sheep 'Dolly' (Wilmut et al. 1973), produced by somatic cell nuclear transfer (SCNT), was a great leap forward for production of not only cloned but also cloned and genetically modified animals. Transgenic animals of various species are today generated for production of essential pharmaceutical products, as animal models for the study of specific human diseases, or for research purposes.

Practical Uses of Assisted Reproductive Technologies

Breeding

One of the major advantages of using technologies such as artificial insemination, embryo transfer, superovulation, and cryopreservation is the breeding aspect, in which the technologies can be used to increase the number of offspring from males and females of high genetic value. In cattle a systematic use of superovulation, embryo collection, and embryo transfer has been used in the so-called MOET breeding programmes, in which young females produced by embryo transfer are used to create large full-sib or half-sib families, from which information is retrieved on a potential breeding bull's sisters rather than his daughters (Fig. 44.1). This will increase the selection intensity and reduce the generation interval and thus the genetic progress. The original MOET programme includes establishment of a central breeding herd, from which all genetic selection is based on records obtained within the herd. A variety of MOET programmes have been introduced from the mid-1980s and the genetic advantage has been demonstrated by this method in a large number of bulls (Callesen et al. 1996).

Horse owners may choose different methods of ARTs for various reasons: multiple offspring from dams and/or sires with high genetic value; offspring from mares that are in competition or terminally ill; or from subfertile mares in whom the cause is not related to problems in the genital tract. Although interest in commercial embryo transfer programmes is still limited, the recent improvements in superovulatory regimens are promising and may change the future demand for embryo transfer. Furthermore, advances in methods for embryo cryopreservation may lead to easier management because there will be no need for recipient synchronisation. Although the American Quarter Horse Association ruled in 2002 to allow registration of multiple foals per donor produced by embryo transfer, several breeding associations still impose limits on the number of such progeny or will not allow registration at all.

With the implementation of ultrasound-guided transvaginal oocyte recovery (ovum pick-up, OPU) and in conjunction with *in vitro* embryo production, it is now possible to use this technology as both a supplement to and a replacement for the traditional *in vivo* embryo production. In this way, embryos may be produced

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from animals with nonheritable infertility (for example, age, occluded oviducts, endometritis), from animals known to have a poor superovulatory response (individual cattle), from prepubertal or pregnant animals, and in the event of sudden unexpected death when ovaries may be recovered from the dead animal and oocytes subsequently processed *in vitro*.

Twinning

In certain beef cattle breeds, twinning may be an attractive prospect. With the transfer of two *in vivo* derived or *in vitro* produced embryos (IVP) to each recipient, it is possible to obtain a calving rate for each embryo that is greater than 100%. Embryos should be placed in both horns, as this will improve the number of fetuses that survive (Sreenan & Beehan 1976). It was speculated that the technology would gain a broader application with the use of IVP embryos, which can be produced at a lower price (Lu & Polge 1992), but it has not been used widely in practice, as it requires expensive equipment and advanced training; in some countries, it is even prohibited by law.

Export and Import

The potential risk of disease transmission with *in vivo* derived and *in vitro* produced embryos is described later in this chapter. It should be emphasised that movement of livestock as embryos is safer and preferable from an animal welfare perspective compared with transport of live animals, whether young or adult. Embryos have also been exported and imported throughout the world to improve the performance of indigenous cattle populations. In addition to a potential genetic gain, embryo transfer into local recipients will also allow the offspring to acquire immunity through colostrum. However, it should be emphasised that the training of people to ensure a high management level is important to achieve the anticipated production gains. In many countries or regions the price of embryos will prevent use of this technology, so artificial insemination using semen from sires of high genetic merit is probably a better and more realistic means of achieving genetic improvement.

Endangered Species

Several ARTs are used as part of the work with conservation of endangered species (Andrabi & Maxwell 2007). However, the number of animals produced is very much lower than for domestic species. This is partly due to scarce and costly animal resources, lack of economic demand, and inadequate research funding, resulting in insufficient knowledge of both the basic reproductive physiology and anatomy in many of the species, as well as the practical handling of the animals and the gametes involved.

Research

Apart from the previously mentioned applications, the development of ARTs has played an invaluable role in understanding basic cell biology events during normal and abnormal fertilisation and embryogenesis, e.g., placental development, ultrastructure, gene expression, chromosomal studies, epigenetics, and metabolism, in particular by comparing *in vivo*-derived embryos and embryos produced *in vitro* or by cloning. The experience obtained from domestic animal species may also be applied in human ART. For example, has the cloning era clearly shown the importance of epigenetic influences on the phenotype? The combination of transgenesis and cloning is now a possible tool for producing animal models for human diseases, thus developing better methods for the diagnosis and means of treatment.

Superovulation, Insemination and Recipient Synchronisation

Purpose

Superovulation, also called superstimulation, is a treatment intended to increase the ovulation rate and thus the number of available oocytes in the donor animal without disrupting the physiological and endocrinological processes associated with oocyte maturation, ovulation, and fertilisation, as well as subsequent embryonic and fetal development. Superovulation is a prerequisite for the successful application of embryo transfer in species with a physiologically low ovulation rate (cattle, sheep/goats, and horses), and the current description of superovulation will focus on these species. A close synchrony of oestrus between the donor and the recipient is crucial for obtaining optimal pregnancy rates and is an important part of the entire embryo transfer planning process.

Hormones

Follicular Stimulation

Two distinct groups of follicle-stimulating hormones have been used for superovulation. The first hormone used to superovulate ruminants was pregnant mare's serum gonadotrophin (PMSG), now designated equine chorionic gonadotrophin (eCG), which is a glycoprotein produced by the endometrial cups of pregnant mares. It is purified from pregnant mare's serum and has an effect that simulates both follicle-stimulating hormone (FSH) and luteinising hormone (LH). After injection in cattle, eCG has a half-life of approximately 5 days, and its use has both advantages and disadvantages. The advantage is that the drug is relatively cheap and is administered only once because of its long half-life. The disadvantages are: first, a variable and unpredictable FSH:LH effect ratio; and second, because of its long half-life, the residual amount of eCG can have a continuous stimulatory effect after ovulation, causing development of postovulatory, oestrogen-producing follicles. This prolonged and elevated oestrogen production may affect fertilisation and early embryonic development, and attempts have therefore been made to reduce the allegedly adverse effects of residual amounts of circulating eCG by injecting eCG antiserum around the time of oestrus. This was found to improve embryo number and quality in some (Dieleman & Bevers 1987) but not all studies (Callesen et al. 1992).

The second group of superovulatory hormones is FSH, a gonadotrophin extracted from the pituitary glands of pigs (pFSH), sheep (oFSH), or horses (eFSH). In contrast to eCG, pituitary gonadotrophin has a half-life of approximately 6 hours and therefore must be administered twice daily for 3 to 4 days in order to obtain the desired effect. Compared with eCG, it is expensive, but most FSH preparations have a more consistent FSH:LH effect ratio. Today, FSH preparations are the most widely used hormone for superovulation.

Induction of Oestrus

To achieve ovulation of the stimulated follicles, the donor must come into oestrus within a few days after the initiation of the eCG or FSH injections, and this requires induction of luteolysis, as the superovulatory treatment normally is initiated in the midluteal phase of the oestrous cycle. Induction of luteolysis is normally achieved with injections of prostaglandin ($PGF_{2\alpha}$) or its analogues, but insertion of vaginal or subcutaneous

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progesterone-releasing devices may also be used to suppress the LH surge until ovulation is desired.

Induction of Ovulation

Two hormones may be used, either human chorionic gonadotrophin (hCG) or gonadotrophin-releasing hormone (GnRH), either used alone or in combination with prostaglandin.

Recipient Synchronisation

Recipients are synchronised with prostaglandin or progesterone-releasing devices, and a close synchrony with the donor's oestrous cycle is always attempted (see Chapter 8).

Superovulation, Insemination, and Synchronisation Regimens in Cattle

A vast amount of data is available concerning superovulation in relation to embryo transfer in cattle (Mapletoft & Bo 2012), and in the following information, the practical importance of this is summarised.

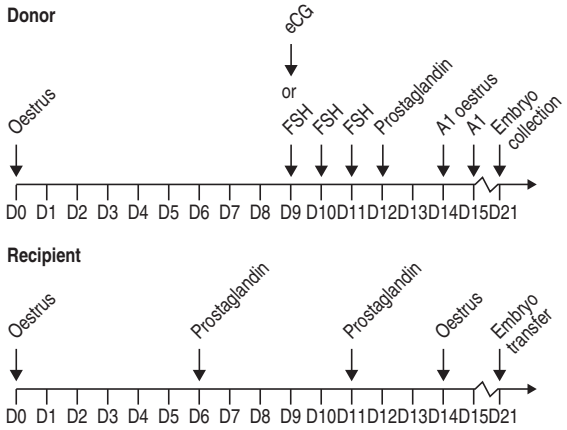
It must be stressed from the onset that superovulation of cattle is subject to a high degree of unpredictability and variability and may cause frustrations for the donor's owner and also for the veterinarian involved (Callesen & Greve 2002). Before entering into an embryo transfer programme, the owner should therefore receive detailed information and veterinary guidance about the shortcomings of superovulation and the procedure as a whole. However, there are a number of routine clinical and management procedures that may be undertaken to increase the likelihood of success.

Donor Selection

One of the routine measures is the careful assessment of potential donors. They should be in positive energy balance, and cows losing weight after calving are particularly poor candidates for induction of superovulation. In general, they should have had two regular oestrous cycles before commencement of the procedure and be otherwise gynaecologically normal. Age, parity, and season (except heat stress) have some influence on the subsequent outcome of superovulation, and there is also a certain breed effect. Ultrasonography may be used before superovulation to ensure that there is a degree of ovarian follicular activity (Singh et al. 2004), but the correlation between the ultrasonographically visualised number of small and medium-sized ovarian follicles at the time of initiation of hormone treatment and the subsequent number of ovulations is not high but may give some indication of the donor's subsequent response (Singh et al. 2004). The blood concentration of anti-Müllerian hormone (AMH) is an indicator of the active follicle population in the donors. It has some potential application as a method in assisting in the selection of donors for superovulation (Baruselli et al. 2015), but variation between breeds and different genetic lines requires further investigations.

Standard Superovulation Protocol

At the time of initiation of the gonadotrophin treatment, the veterinarian must ensure that there is an active CL. The standard superovulation protocol (Fig. 44.2) is as follows: around Day 9 to 11 after oestrus/ovulation, when the second follicular wave is emerging, the donor receives one injection of eCG or the first of FSH administered twice daily in decreasing doses for 3 to 4 days. At 48 to 72 hours after the initial injection of the superovulatory treatment, oestrus is induced by means of one or two injections of prostaglandin, and oestrus will normally occur around



• **Fig. 44.2** Hormonal regimen for superovulation and oestrus synchronisation in cattle.

48 hours afterwards. Because oestrus may occur earlier because of the increased oestrogen production from the growing follicles, it is advisable to observe the donor closely for oestrus signs from around 36 hours after prostagandin treatment. The LH surge coincides with the occurrence of oestrus, namely 42 to 45 hours after the prostaglandin injection (Callesen et al. 1986), and the donor will ovulate 24 to 30 hours later. Therefore insemination should be performed twice, at 12 and 24 hours after the onset of standing oestrus; it has been shown that more inseminations have no advantage (Callesen & Greve 2002).

It is important to use high quality semen (Stroud & Hasler 2006) from bulls of known fertility because semen transport in the superovulated female may be impaired; this is shown by the relatively high rate of unfertilised oocytes and the low number of supernumerary spermatozoa in the zona pellucida of embryos recovered from superovulated animals, compared with nonstimulated animals (Saacke et al. 1994). A certain bull effect has been seen (Callesen & Greve 2002), and there is also a farm-to-farm variation that is probably related to the farm management. When sex-sorted semen is used, factors such as inherent variation in the response of a particular bull's semen 'being sorted', the dose of sexed semen used for insemination, the insemination protocol, and the parity of the donor are also to be considered (Peippo et al. 2009).

Recipient Selection and Management

A close synchrony between donor and recipient oestrus is essential to achieve high pregnancy rates (Hasler 1998). To induce oestrus in the recipients, they are given two doses of prostaglandin 11 days apart (see Chapter 8). The last injection is given 24 hours before the donor's prostaglandin injection, as the recipient has a longer response time to oestrus in comparison to the donor. The reason for this is the earlier and increased rise in the donor's oestrogen (E2) concentrations that will trigger an earlier LH surge.

Measures to Improve the Superovulatory Response

Many attempts have been made to improve the superovulatory response after the conventional midcycle superovulatory regimen, and one focus area has been the follicular wave pattern through the oestrous cycle assessed by ultrasonography (Adams 1994, Ginther et al. 1996). This has helped in the design of new and more effective protocols for the manipulation and control of the follicular wave emergence (Mapletoft & Bo 2012).

Taking into account the pattern of follicular wave emergence, it is possible to design protocols in which the start of the FSH

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treatment coincides with the time of the wave emergence and when ovulation is timed so fixed-time AI can be performed (Callesen et al. 1992). This reduces the time spent by staff involved and also removes the need for oestrus detection; it furthermore optimises the number of fertilised eggs obtained (Bo et al. 2006). However, such protocols are not possible to use in several countries, particularly in the EU, where there are restrictions on the use of certain hormones in animals intended for human consumption.

Recombinant bovine somatotropin (rbST) has been shown to increase the number of growing follicles in cattle (Gong et al. 1993) and thus, theoretically, could be used to improve the superovulatory response. However, using repeated superovulations in a commercial organisation involving embryo transfer in dairy cattle (Hasler et al. 2003) failed to demonstrate a positive effect of this treatment in terms of total number of embryos and those of good quality.

Disadvantages and Variability of Superovulation Treatment

Superovulation has at least two major challenges: first, the efficacy of the gonadotrophins (purity, FSH:LH effect ratio) and the complex planning and management of the superovulatory protocol in relation to the emergence of the follicular wave; second, there exists an inherent between-animal variation in terms of the number and quality of both oocytes and embryos produced.

Superovulation always results in aberrant steroid hormonal profiles in both the follicular fluid and the peripheral circulation (FSH, LH, E2, and P4). Thus there are significantly elevated levels of E2 and P4 around the time of and after ovulations, and in most donors the LH surge is elicited sooner due to an earlier increase in E2. In some donors the LH peak is lower or absent. All or just some of these aberrations may lead to impaired oocyte maturation, reduced oocyte quality, lack of fertilisation, and an impaired embryo quality (Callesen et al. 1986). The only hormonal analysis that may be of practical relevance is the measurement of plasma P4 around the time of oestrus, as elevated levels are predictive of a poor response (Dieleman & Bevers 1987, Callesen et al. 1992, Callesen & Greve 2002).

The Donor After Superovulation and Flushing

Within approximately 30 days for heifers and 40 to 60 days for cows, the donors will return to normal cyclicity, and their fertility is generally normal. Nevertheless, through the profound alterations in the endocrine profiles, superovulation may obviously affect the subsequent fertility of the donor for a period of time, and it is important that the owner is made aware of this. Embryos may be left in the uterus after flushing, and to avoid spontaneous abortion around day 50 to 60 of multiple fetuses and to achieve a return to normal cyclical activity as soon as possible, prostaglandin should be given at the end of the collection procedure and eventually repeated some days later. Nevertheless, a certain proportion of animals will not return to normal cyclicity, as they may experience an anovulatory period or even develop cystic ovarian disease. These donors should be submitted for gynaecological examination, and treatment with progesterone may alleviate the problem.

Superovulation, Insemination, and Synchronisation Regimens in Horses

Superovulation

In the past, attempts to induce multiple ovulations in the mare have resulted in only marginal success. Paradoxically, eCG is ineffective in inducing a superovulatory response in the mare (Day 1940, Betteridge & Mitchell 1974), presumably because of insufficient binding to the FSH receptors in the mare's ovarian tissue (Stewart & Allen 1979). Different treatment regimens with pFSH have only resulted in an insignificant increase in the average ovulation rate of fewer than 2 ovulations, even when quite large doses were used (Irvine 1981, Squires et al. 1986, Fortune & Kimmich 1993). GnRH or GnRH-analogue treatment of mares during anoestrus has a moderate superovulatory effect (Johnson 1987, Ginther et al. 1990), whereas in cyclical mares, GnRH treatment has no effect and results in downregulation of the pituitary gland, with ovarian inactivity as a consequence (Montovan et al. 1990, Watson et al. 1992, Fitzgerald et al. 1993, Pedersen et al. 2002). Active immunisation against the α-subunit of inhibin has been used successfully to increase the ovulation rate, although passive immunisation only had a moderate effect. Immunoglobulins against inhibin neutralise the circulating inhibin and reduce the inhibitory effect on FSH from the pituitary. However, this method has not been used commercially because of variable results, the need for repeated injections, and several adverse reactions experienced after both active and passive immunisation (McCue et al. 1993, McKinnon et al. 1992).

It has been hypothesised that the relatively restricted area available in the ovulation fossa, combined with the large size of the preovulatory follicle in the mare, might be a limiting factor for superovulation (McCue 1996). Furthermore, if blood clotting in the ovulation fossa is increased when multiple ovulations occur on the same ovary, it may lead to lower embryo recovery rates when double ovulations occur on the same ovary as opposed to one follicle on each ovary (Squires et al. 1987, Riera et al. 2006). However, a report in which a mare spontaneously ovulated 12 follicles of diameters ranging between 18 and 22 mm within 2 days, followed by ultrasonographic identification of six embryonic vesicles on gestation Day 17 (Brück et al. 1997), shows that there is no intrinsic limitation preventing superovulation in this species. Although the mechanism for this spontaneous superovulation has never been determined, it clearly illustrates that the diameter of the preovulatory follicle is not necessarily a measure of follicular and oocyte maturity and that the normal mechanisms for selecting a dominant single follicle may sometimes be overridden.

Successful superovulation, with ovulation rates ranging from 1.7 to 4.6 ovulations/oestrus, has been achieved using equine pituitary extract (Squires et al. 1986, Douglas et al. 1974, Woods & Ginther 1984). Currently, the most effective protocol to induce multiple ovulations in the mare is a regimen with twice-daily injections of FSH (eFSH or recombinant FSH), initiated 5 to 8 days after ovulation when a CL is present and the largest follicle has reached 20 to 25 mm in diameter. On the second day of FSH treatment, prostaglandin is administered to induce luteolysis. The FSH treatment is continued for 3 to 5 days until the largest follicle(s) reaches 32 to 35 mm in diameter. After an interim period of 36 hours, the mare is treated with hCG to induce ovulation (Alvarenga et al. 2001, Scoggins et al. 2002, McCue et al. 2007, Meyers-Brown et al. 2011). This regimen has resulted in an average of approximately four ovulations and 1.5 embryos recovered per treated oestrous cycle (Niswender et al. 2003, Welch et al. 2006). The relatively intensive treatment regimen and the current price per dose render superovulation treatment in the mare an expensive and impractical procedure. As in cattle, there is variability in the superovulatory response, especially in older mares (Squires & McCue 2007), and so far, there is no information available on the effect of repeated superovulatory treatments on the mare's long-term fertility.

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Synchronisation and Insemination

For oocyte and embryo transfer, the time of ovulation has to be synchronised between donor and recipient mare. This is generally attempted by two separate injections of prostaglandin during dioestrus to induce luteolysis, followed by treatment with hCG or GnRH analogue during oestrus when the preovulatory follicle reaches 35 mm in diameter in order to induce ovulation. Nevertheless, the course of follicular development varies considerably between individual mares, and the onset of oestrus depends greatly on the size of the follicles present at the time of prostaglandin injection. Thus simple synchronisation with one prostaglandin injection during dioestrus is often suboptimal, and the administration of two prostaglandin injections approximately 10 days apart can improve the synchrony; alternatively, three recipients should be available per donor mare. Timed induction of luteolysis can also be combined with a previous prolonged treatment with allyltrenbolone (Loy et al. 1998), which has been shown to have the lowest incidence of adverse reaction compared with other forms of progestin treatments (Card et al. 2015). Fortunately, the degree of synchrony between the oestrous cycles of donor and recipient is less crucial in mares than in cattle. No significant difference in the pregnancy rates after transfer of embryos collected on Day 7 after ovulation was found if the recipient ovulated 1 day before or up to 5 days after the donor mare, although there was a trend for better pregnancy rates if the recipient ovulated 2 or 3 days after the donor (Squires et al. 1982, Jacob et al. 2012). Alternatively, pregnancies and foalings can be achieved after embryos are transferred to ovariectomised recipients that are kept under progesterone treatment for the first trimester of gestation (Hinrichs & Kenney 1987). This method has a major practical implication for commercial embryo transfer in mares because embryo transfer programmes are often hampered by the unreliability of recipient synchronisation and the unavailability of sufficient numbers of recipient mares.

The breeding technique (natural service or AI) is generally secondary, provided semen quality and the timing of breeding in relation to ovulation is optimal. If there is uncertainty concerning either the quality of frozen semen or the possibility of intensive monitoring necessary for AI with frozen semen, the use of fresh or chilled semen is preferred.

Superovulation, Insemination, and Synchronisation Regimens in Sheep and Goats

Superovulation

Techniques for superovulation are very similar for both species (Ishwar & Memon 1996), and the gonadotrophin preparations used are the same as those used in the cow, namely eCG or oFSH. Gonadotrophin treatment is usually initiated in mid- to late cycle, and prostaglandin is administered 24 to 72 hours later, inducing oestrus within 24 to 36 hours. Oestrus and ovulation can also be controlled by an intravaginal progesterone-releasing device; sheep are treated for 12 to 14 days and goats for 14 to 18 days using this method. Superovulation can also be induced outside the normal breeding season. The use of eCG antibodies has been attempted in sheep but without significant improvement of the superovulatory result.

Insemination

Insemination is usually achieved by natural service or AI using freshly collected semen every 12 hours from the time of oestrus. However, fertilisation failure occurs commonly in the ewe, particularly when the ovaries are overstimulated and response to gonadotrophin is high. This can be overcome by surgical insemination directly into the uterus, either by laparotomy or using laparoscopy. Synchronisation of donor and recipient oestrous cycles is normally achieved by progesterone-releasing intravaginal sponges, and an asynchrony of up to 2 days will still result in acceptable pregnancy rates (Ishwar & Memon 1996).

Superovulation, Insemination, and Synchronisation Regimens in Pigs

Superovulation and Insemination

Induction of superovulation of sows or of gilts involves the use of two very different regimens as described here.

In sows, hCG is administered 3 days after weaning to induce ovulation, and 15 days later luteolysis is induced by means of two prostaglandin injections given 12 hours apart (Hazeleger et al. 1999). To stimulate follicular growth, eCG (1000 or 1500 IU) is administered approximately 1 day after the last prostaglandin injection. Ovulation is induced 72 hours later with hCG (750 IU), and the sows are inseminated 36 and 42 hours after hCG injection. Recipients receive the same treatment apart from insemination.

In gilts, there are two possible methods: (1) insemination and induction of abortion with prostaglandin around Day 26, and subsequent superovulation with eCG; or (2) use of allyltrenbolone in combination with eCG and hCG (Cameron et al. 2004). In the latter method, after a natural oestrous cycle, the pigs are fed allyltrenbolone (20 g/day) for 18 days and, 24 hours after cessation of this treatment, they receive an injection of 1000 IU eCG followed by 750 IU hCG approximately 72 hours later. Mating (natural or artificial insemination) is then performed two or three times, starting approximately 24 hours after hCG. The latter method is preferred.

Embryo Collection and Transfer

Purpose/General Principles

After superovulation, the embryos are recovered from either the oviduct or the uterus. In all species, early attempts to recover embryos used surgical methods through a midventral laparotomy (Polge 1977) and flushing of the oviducts and uterus. This technique is still practised in species in which cervical catheterisation is difficult, and the procedure cannot be controlled by rectal palpation (sheep/goats and pigs). As a consequence, it is labour intensive, requires access to an operating theatre, is expensive, and poses a greater risk to the donor animal (anaesthesia, adhesions, and subsequent reduced fertility). A semisurgical technique involving endoscopy for the recovery of embryos from the oviduct has been developed both for pigs and cattle (Besenfelder 2006); its use is currently for more experimental purposes.

In cattle and horses, nonsurgical techniques were developed to collect embryos from the uterus of cattle (Drost et al. 1976, Elsden et al. 1976, Greve et al. 1977, Newcomb et al. 1978) and horses (Oguri & Tsutsumi 1974, Imel et al. 1981). This was a major advance in the application of commercial embryo transfer, especially in cattle.

Embryo Collection and Transfer in Cattle

Embryo Collection

Bovine embryos enter the uterus around Day 4 and are collected 6 to 8 days after the onset of standing oestrus when the embryos

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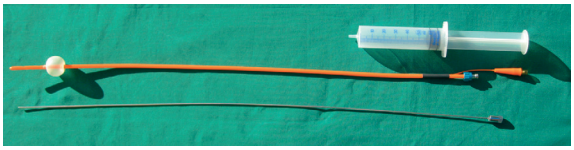
• **Fig. 44.3** Cattle ovary with multiple corpora lutea after superovulation with eCG.

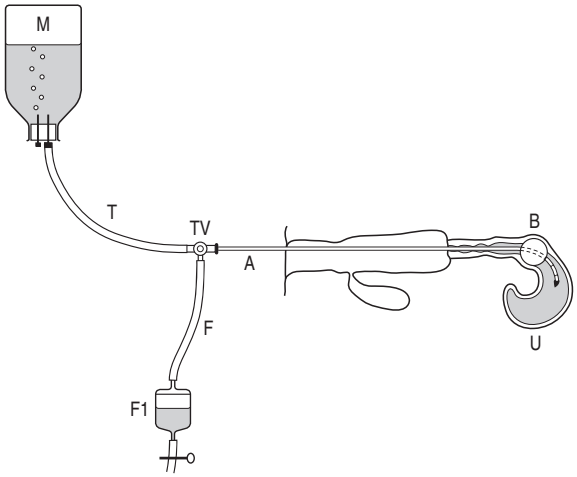


• **Fig. 44.4** Foley catheter used for nonsurgical embryo collection in cattle, balloon extended with air.

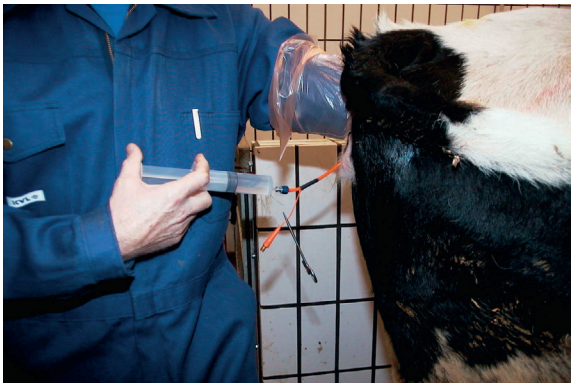
are at the morula and blastocyst stages and still surrounded by the zona pellucida (see Chapter 4).

Before embryo collection, the superovulatory response is assessed by rectal palpation (Fig. 44.3) and/or use of transrectal ultrasonography; the latter method gives a more accurate estimate of the number of CLs and anovulatory follicles. However, the exact number of CLs is difficult or even impossible to assess, particularly in donors with multiple CLs, and the veterinarian should avoid telling the owner about the expected number of embryos. If there are only one or two CLs or there are follicular cysts, it is advisable not to proceed with the collection procedure. Epidural anaesthesia is normally used to prevent straining and defecation. However, some animals may aspirate air into the rectum (ballooning) as a consequence of the epidural, and this makes the procedure more difficult. It is important to minimise contamination, so the perineal region is washed and disinfected carefully with a mild disinfectant, including the inside of the vulvar lips.

The embryo collection catheter most widely used is a Foley rubber catheter (Fig. 44.4) of different brands and sizes; a catheter 70 cm long and 14, 16, or 18G is most commonly used for cows and heifers, respectively. The catheter is guided through the cervix into the desired uterine horn under careful rectal control, and when the tip of the catheter is in the middle part of the horn, the balloon is inflated with air or saline. Care should be taken not to damage the endometrium during the passage of the catheter and during the inflation procedure, as this may lead to poor recovery of the flushing medium and/or bleeding with subsequent clotting, leading to difficulties in embryo identification. In heifers, it may be necessary to use a cervical dilator before introducing the catheter, but care must be taken not to penetrate the uterus just after passing through the last cervical ring.



• **Fig. 44.5** Schematic drawing of nonsurgical embryo collection in cattle using continuous flushing by the gravity flow.



• **Fig. 44.6** Embryo recovery in a heifer using the interrupted flow principle.

It is customary to flush each horn separately; flushing of the whole uterine body can be used but often requires more uterine manipulation than the separate horn flush. Modified Dulbecco's phosphate buffered saline (DPBS), supplemented with 1% to 2% bovine serum albumin (BSA) or fetal calf serum (FCS) and various antibiotics, were normally used for recovery (Greve et al. 1977); however, today, many companies sell ready-to-use media for this procedure. Protein supplementation of the flushing medium is important in order to prevent adhesion of the embryos to the plastic ware (filters, dishes, and tubing), but it is essential to assure that the protein source is not contaminated with virus (e.g., bovine viral diarrhoea (BVD)). Two different embryo collection (flushing) methods may be used: continuous flow (Fig. 44.5) or repeated interrupted collection using injection and evacuation of 30 to 50 mL medium (Fig. 44.6). The flushing volume varies between animals, but in general, approximately 0.5 litres is used per horn, with cows requiring more than heifers. Whatever method is used, it is of the utmost importance to recover all of the medium that has been infused, as the embryos may be present in the final residues.

The flushing medium is transferred to a specially designed filter with a pore size of 70 µm, allowing somatic cells (e.g., red blood cells) and certain debris to pass through but retaining the embryos (150–200 µm in diameter). The filter content is transferred to a dish marked with squares, and the filter bottom is washed several times with additional flushing medium. Sedimentation in a funnel

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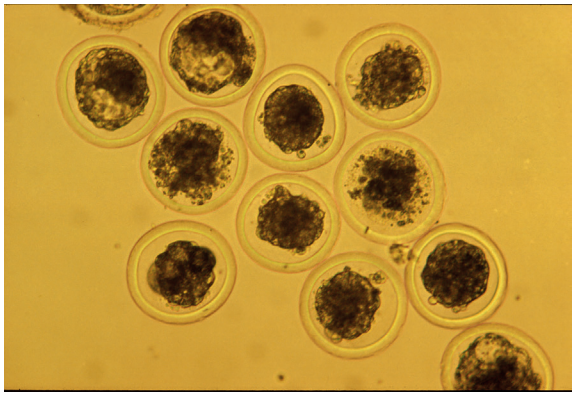

• **Fig. 44.7** *In vivo*-produced cattle embryos recovered on Day 6 to 7 after oestrus.

or glass cylinder can be used, but mucus and other debris may mask the embryos during the subsequent search in the dish. The search is performed under a stereomicroscope at ×50 to 100 magnification and, once the embryos are identified, they are transferred to a smaller dish containing the appropriate medium, e.g., DPBS medium supplemented with 5% to 10% FCS or another protein source.

Assessment of Embryo Quality

Embryo evaluation is performed in a dish that should contain no blood or other debris. The morphological evaluation of embryos includes assessment of stage and quality of the embryo and follows the guidelines from the International Embryo Technology Society (Stringfellow & Givens 2010). Among the fertilised eggs recovered on Day 6 to 7, most embryos are at the compact morula, early blastocyst, blastocyst, or expanded blastocyst stages (Fig. 44.7). Hatched blastocysts will rarely be present if collections are conducted before Day 8 after oestrus. A compact morula contains at least 16 cells, and the surface of individual blastomeres may be discerned. In the early blastocyst there is a small blastocoele cavity, so it is often possible to distinguish the inner cell mass and the trophectoderm. During the observation in the dish, the embryos may 'pump' and form a smaller or larger blastocoele cavity; it is believed that this is a sign of good health and quality. The embryo quality is scored as excellent, good, fair, or poor; additionally unfertilised oocytes have to be identified. It may be difficult to make a very clear distinction between excellent and good quality embryos, which are characterised by the embryo being spherical without signs of blastomere degeneration. In the fair category, some blastomeres may have degenerated and are lying outside the trophectoderm, so they are not participating in the embryo formation. In the poor category, very few blastomeres are normal. The unfertilised oocytes often assume different shapes and sometimes contain degenerated, unevenly distributed ooplasm.

In vivo-derived bovine morulae and blastocysts are quite robust and may be maintained in the appropriate medium in a small dish or in an AI straw at room temperature for up to 24 hours without losing their viability. If stored at 4°C, transfer will result in satisfactory pregnancy rates even after storage up to 48 hours after recovery (Lindner et al. 1983). Moreover, *in vivo*-derived embryos are quite tolerant to cryopreservation (see later in this chapter).

The results of embryo recoveries are subject to large variations (Stroud & Hasler 2006, Hasler 1998) as they depend on the response to superovulation. Nevertheless, in general, one may expect that:

- Five percent of the donors are not flushed because of a poor superovulatory response, i.e., 1 to 2 CLs.
- Nontransferable or nonviable embryos are recovered from 20% of the animals.
- The total number of embryos and unfertilised oocytes per donor is around 8 to 10, and of these, approximately 6 embryos are considered to be transferable (Stroud & Hasler 2006, Merton 2007, Mapletoft & Bo 2012).

Embryo Transfer

The embryo may either be transferred to the recipient immediately after recovery or be cryopreserved and transferred later. Data from the International Embryo Technology Society show that approximately 40% of the transferred embryos are fresh, whereas the other 60% are first cryopreserved (Perry 2016). Quite many of the cryopreserved embryos are not subsequently thawed and transferred, for example, because of lack of demand resulting from decreasing genetic value of these embryos after too long storage.

Until the mid-1970s, embryos were transferred surgically initially by midventral and later by flank approach into the tip of the uterine horn. These techniques – in particular the midventral approach – were tedious and involved a surgical risk to the animals. Since then, virtually all bovine embryos have been transferred by nonsurgical means through the cervix, yielding pregnancy rates similar to those obtained after surgical transfer, i.e., on average, 50% to 60% (Greve & Lehn-Jensen 1979, Wright 1981).

Before transfer, the embryos are washed 10 times according to the manual of the IETS (Stringfellow & Givens 2010), and each embryo is then loaded into a 0.25 mL sterile insemination straw. The straw is placed in an ordinary insemination pipette or a specially designed embryo transfer catheter, which is a thinner and longer version of the ordinary insemination pipette.

The oestrous cycle of the recipients must be synchronised with that of the donor, and before transfer, it must be ascertained that oestrus has occurred within ± 24 hours of the donor (Hasler 1998) and that a functional CL is present. The synchrony between recipient and embryo developmental stage may be a more reliable parameter than oestrous synchrony. Thus morulae should, for example, be transferred to recipients that are at Day 6 after oestrus, and blastocysts to recipients at Day 7.

It is advantageous to use epidural anaesthesia. However, this can be omitted, for example when the transfers are done by nonveterinarians who are not allowed to use anaesthetic drugs. As with the donors, the perineal region has to be cleaned carefully. In cattle, embryos must always be transferred to the uterine horn ipsilateral to the ovary bearing the CL in order to block the luteolytic mechanism. The embryo transfer catheter, fitted with a chemise, is passed through the cervix under rectal control and gently introduced into the midpart of the uterine horn, where the embryo is deposited (Fig. 44.8). The technique involves a combination of gently pushing the catheter into the uterine lumen and pulling the horn over the catheter. Any endometrial trauma with bleeding must be avoided. In terms of pregnancy rates (average 50%–60%), there are considerable variations from operator to operator and from farm to farm, and there is also a very significant effect of embryo quality, with excellent and good embryos giving the highest pregnancy rates.

After embryo transfer, pregnancy loss beyond gestation Day 45 is around 5% and thus very similar to that after artificial insemination, with cryopreserved poor quality embryos resulting in the highest loss (Callesen et al. 1996).

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• **Fig. 44.8** Embryo transfer procedure in a heifer using an embryo transfer catheter fitted with a chemise.

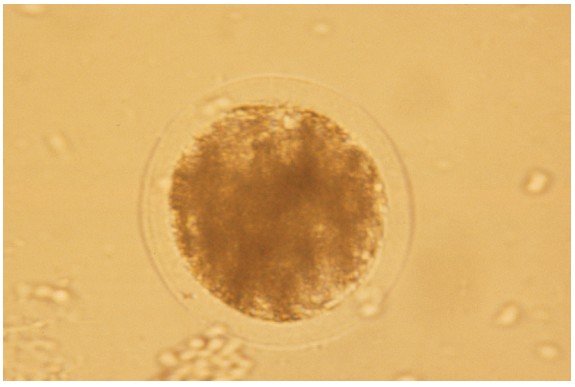
• **Fig. 44.9** Nonsurgical embryo collection in a mare on Day 7.

Embryo Collection and Transfer in Horses

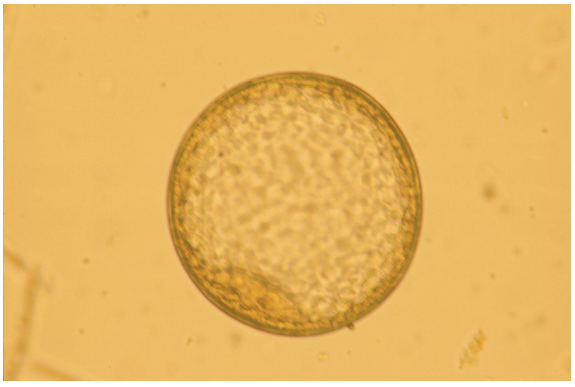
Embryo Collection

For comfortable working conditions, mares are restrained in a crush, and sedation is often unnecessary (Fig. 44.9). An initial thorough cleaning of the perineal area is essential. The tip of a Foley or silicone flushing catheter is inserted by digital manipulation through the cervix into the uterine body. The balloon of the catheter is inflated just cranial to the cervix, and the entire uterus is filled by gravity flow with Dulbecco's PBS supplemented with 1% FCS, which prevents especially the smaller embryos from adhering to the plastic ware. The volume of the flushing medium varies with the mare's age and parity, but in general, the uterus will accept 1 to 2 litres. It is important to install as much fluid as the uterus will accept, to ensure that all endometrial folds are distended before the flushing medium is collected through a 70 µm filter. Flushing is repeated three times, and oxytocin may be given before the last collection to enhance the recovery of the remaining fluid. Unlike cattle, the recovered flushing medium rarely contains red blood cells. Immediately after recovery, the donor can be treated with prostaglandin to induce oestrus and allow embryo recovery to be repeated with a 14- to 18-day interval.

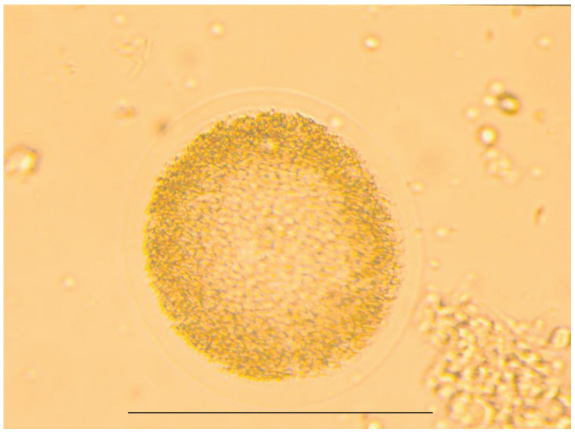
In mares, the embryo enters the uterus at the morula stage, approximately 5.5 days after ovulation (Betteridge et al. 1982), and



• **Fig. 44.10** Equine morula recovered *in vivo* on Day 7 after ovulation.



• **Fig. 44.11** Equine blastocyst recovered *in vivo* on Day 7 after ovulation.



• **Fig. 44.12** Unfertilised ovum recovered from a mare together with a fertilised egg.

in an embryo transfer programme, embryos are generally recovered 6 to 8 days after ovulation at the morula or blastocyst stage (Figs 44.10–44.12).

On Day 6, just upon entry into the uterus, the embryo has a similar size to unfertilised oocytes (approximately 150–250 µm in diameter (Betteridge et al. 1982)), but as the blastocoele forms, it expands rapidly to measure 200 to 600 µm in diameter on Day 7 and up to 600 to 1000 µm on Day 8 (Betteridge et al. 1982). This allows macroscopic identification of the embryo in the flushing medium from approximately Day 8. At this stage the zona pellucida has been shed, so the embryo is now surrounded by a capsule,

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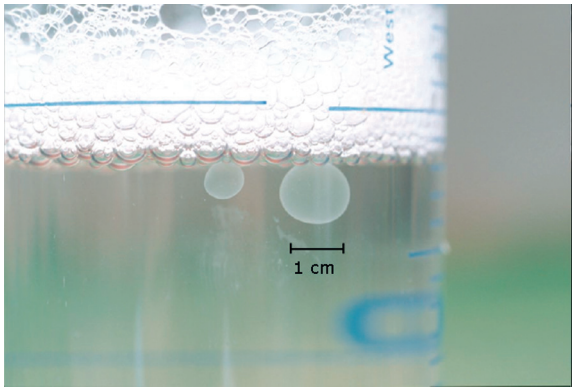
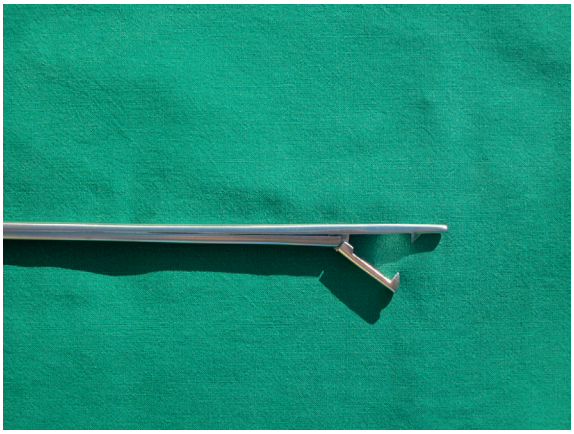


• **Fig. 44.13** Twin equine embryos recovered simultaneously on respectively Day 11 and 14 after asynchronous ovulation. Note that both embryos are floating at the surface indicating a lower specific gravity than the flushing medium.



• **Fig. 44.14** Cervical forceps used for embryo transfer in the mare.

which preserves its spherical shape until approximately gestation Day 17 (Fig. 44.13). Because the passage through the uterotubal junction requires a signal from the early embryo, most probably PGE2 (Weber et al. 1991), recovery of an unfertilised oocyte is always a sign that a fertilised ovum has also passed through the uterotubal junction.

Embryo recovery rates are correlated with embryonic age, with usually higher recovery on Day 8 than on Day 6 (Iuliano et al. 1985, Squires et al. 1985a, Squires et al. 1985b), most likely due to the larger size and better visibility of the embryo. In young fertile mares, embryo recovery rates of up to 70% to 80% can be achieved on Day 7, but lower recovery rates are usual when working with mares in practice. Thus pregnancy rates after transfer vary greatly, depending on the fertility of the donor, stallion, and recipient, as well as the operator's technical skills; also, the mare's age has a significant influence. Apart from an increased pregnancy loss (reviewed by Ball (1988)), the age-related decrease in mare fertility can be attributed to prolonged follicular development, decrease in oocyte quality, and endometrial alterations resulting in a decrease of the microcotyledon surface density (Brinsko et al. 1995, Carnevale & Ginther 1995, Wilsher & Allen 2003). Thus requirements for the donor are: optimal fertility, 3 to 10 years of age; first or second parity to assure optimal uterine capacity and function; adequate body size and nutritional status; and good temperament. Needless to say, embryo quality plays a major role, and morphological evaluation using light microscopy before transfer may provide an indication of the embryo's subsequent developmental competence. Nevertheless, morphological embryo quality will not always be accurately predictive and due to limited use of superovulation, often only one embryo will be available for transfer.

Embryo Transfer

As for the donor, there are preferable requirements for the recipient with respect to age, parity, fertility, and temperament. As mentioned before, synchrony between stages of the oestrous cycle of donor and recipient should be within + 1 and −5 days. Thus in countries with well-developed commercial horse breeding activities, large recipient stations have evolved. Alternatively, ovariectomised mares treated with progesterone during the first trimester of gestation have proved to be useful recipients (Hinrichs & Kenney 1987, Squires et al. 1989).

Equine embryos can either be transferred to a recipient immediately after recovery, after cooling to 5°C for 24 hours, or after cryopreservation (see later in this chapter). For nonsurgical transfer, the embryo is aspirated into an insemination straw, and for ease of identification, positioned between an air bubble and a second fluid column. The transfer instrument is introduced into the synchronised recipient in a way that in principle is similar to that used for AI but, to ensure extra sterility, thorough cleaning and disinfection of the recipient's perineal area and coverage of the transfer instrument with a sterile chemise (guarded method) are essential. As an additional measure to ensure sterility and avoid prostaglandin release, it has been suggested that the cervix is retracted caudally using cervical retraction forceps (Fig. 44.14) inserted into the vagina inside a Polansky speculum, before the tip of the insemination catheter is introduced through the cervix; this removes the need for manual intravaginal manipulation (Wilsher & Allen 2004). When the tip of the insemination catheter is positioned correctly in the uterine body, the embryo is gently released.

After nonsurgical transfer of Day 7 and 8 embryos, pregnancy rates of 50% to 75% can be achieved (Iuliano et al. 1985, Squires et al. 1999).

Embryo Collection and Transfer in Sheep and Goats

Embryo Collection

In the ewe and the doe goat, embryos are surgically collected 3 to 7 days after oestrus under general anaesthesia and through a midventral laparotomy (Moore 1982). The animals are placed in dorsal recumbency, preferably on a table that allows the hind part of the animals to be elevated (Fig. 44.15). After access to the abdominal cavity, the ovary, oviduct, and part of the uterine horn are exteriorised and the CL number determined in order to predict likely embryo recovery rates. Using a blunt needle fitted on a syringe, approximately 50 mL of, for example, modified DPBS saline containing 1% FCS is infused either into the tip of the horn or through the oviduct, and the medium is collected in dishes via a collection catheter placed in the distal part of the horn. Procedures for identification, isolation, and quality assessment are very similar to those previously described for cattle. The recovery rate is generally between 90% and 100%, and the number of collected embryos varies between and within breeds. Approximately 3 to 10 embryos per donor can be expected. Attempts to use semisurgical techniques (laparoscopy) for embryo recovery of tubal-stage embryos have been made, with similar recovery rates compared with surgical methods (Besenfelder 2006).

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• **Fig. 44.15** Laparoscopic collection of goat embryos.

Embryo Transfer

In goats and ewes, embryo transfer is performed under general anaesthesia using either a midventral laparotomy or laparoscopy. A short period of general anaesthesia is generally used without the need for endotracheal intubation, and it is important that the animal's rear end is elevated, particularly when using the laparoscopic approach. It seems that laparoscopy is as effective as laparotomy, but it requires considerably more experience to obtain good pregnancy rates.

Embryo Collection and Transfer in Pigs

Embryo Collection

Reviews of the principles for porcine embryo collection and transfer have been published by Polge (1982), and it is a technique that has not changed much over the years. Embryos are collected 3 to 7 days after oestrus under general anaesthesia through midventral laparotomy, and after having gained access to the abdominal cavity, the following procedure is performed bilaterally:

- The ovary, oviduct and uterine horn are exteriorised, and a specially designed curved glass tube or a collection catheter is inserted into the uterine lumen approximately 50 cm from the uterotubal junction.
- The medium, e.g., Dulbecco's PBS supplemented with 1% FCS or a commercial product, is flushed through the oviduct using a blunt needle inserted into the ampullar region.
- The fluid is gently massaged through the uterine horn and then collected into dishes via the glass tube or the collection catheter.
- Identification and isolation are very similar to the procedures described for cattle, but in terms of embryo quality, either good quality embryos or some unfertilised oocytes are found or only unfertilised oocytes.

Because of the higher lipid content in the cytoplasm, porcine embryos are darker than their bovine counterparts; moreover, a greater number of accessory sperm heads are found within the zona pellucida. The recovery rate is generally very high, and 25 to 30 embryos are normally harvested at each collection.

Embryo collection may also be performed using a laparoscopic technique (Besenfelder et al. 1997), essentially for experimental purposes. Transcervical embryo collection (nonsurgical) has also been described (Hazeleger 1999), but it requires prior resection of the major part of the uterus and results in low recovery rates. Therefore the only realistic approach for collection of embryos from donor sows is either by surgery or after slaughter (Martinez et al. 2017).

Embryo Transfer

Embryos are usually transferred surgically using a midventral laparotomy to exteriorise the tip of the uterine horn, into which 20 to 30 embryos are deposited. Because they will subsequently migrate into the contralateral horn, bilateral transfers are not necessary (Dzuik et al. 1964). The pregnancy rate is generally around 70% and embryo survival around 60% to 65%, yielding a litter size of five to seven piglets.

Embryos may also be transferred to the uterine tubes of gilts by means of laparoscopy (Besenfelder et al. 1997). The pregnancy rate after endoscopic transfer can be expected to be from 60% to 80%, with around five to six implantation sites.

A transcervical embryo transfer technique was developed for sows (Hazeleger & Kemp 1999) in which a specially designed embryo transfer catheter is placed deep into the uterine horn. The pregnancy rate depends on the stage of development at which the embryos are transferred, with morulae combined with blastocysts yielding a significantly higher rate (55%) than transfers of morulae (10%). The overall pregnancy rate is about 30%, with a litter size of around six to seven piglets. A further modification of this technique has been used in gilts and sows, in which the catheter can be inserted into the uterine horn in 70% to 80% of gilts or sows, resulting in farrowing rates around 80% and litter sizes around 9.5 after transfer of 25 to 30 fresh morulae and blastocysts (Martinez et al. 2013). The subsequent fertility of these recipients is not affected (Martinez et al. 2017).

Oocyte Collection by Use of Ultrasound

Purpose

If IVF is to be considered as a management tool in commercial livestock breeding programmes or as a routine treatment of sub- and infertility, it requires to be supplemented with a technique that allows repeated recovery of multiple oocytes from selected individuals without jeopardising their health and future fertility.

In the past, oocytes have been recovered from live cows and mares, primarily for basic research, using laparoscopy, laparotomy, colpotomy/ovariectomy, or blind needle penetration via the paralumbar fossa (Hinrichs & Kenney 1987, Lambert et al. 1986, McKinnon et al. 1986, Palmer et al. 1987). Obviously, a surgical approach can only be used on a limited number of occasions in each animal; however, the introduction of real time ultrasonography to the veterinary field in the late 1980s played an important role in enabling oocyte collection repeatedly from the same individual. Initially, transcutaneous ultrasound guidance was used to monitor paralumbar puncture (Callesen et al. 1987), but soon the technique of transvaginal ultrasound-guided follicular aspiration, which was first developed in humans, was modified and successfully applied to aspirate oocytes from cattle and mares (Pieterse et al. 1991, Brück et al. 1992, Cook et al. 1992).

Transvaginal ultrasound-guided oocyte aspiration (TVOR), transvaginal follicular aspiration (TVA), or ovum pick-up (OPU) is a rapid and minimally invasive technique for retrieving oocytes repeatedly from a donor animal. Thus it allows either the collection of oocytes for IVP (cattle) or oocyte transfer (mares) from genetically valuable animals and also if they would normally be considered infertile because of uterine diseases, oviductal occlusions, or a poor superovulatory response. Circumventing heritable infertility must obviously be avoided.

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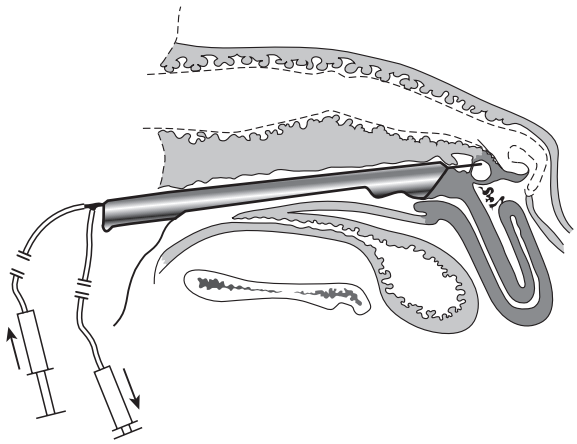

• **Fig. 44.16** Schematic drawing of transvaginal–ultrasound-guided follicular aspiration in the mare.

Technique

OPU is generally performed in cattle under epidural anaesthesia (occasionally combined with sedation) and in mares after sedation and analgesia. Basically, the technique requires a 5 or 7.5 MHz transducer incorporated into a needle guide that can be inserted into the vagina of a cow or mare. Various models of needle guide and aspiration system have been developed, often based upon the different available ultrasound machines. The ovary is grasped transrectally by the hand and gently pulled caudally towards the transducer head (Fig. 44.16). When a suitable follicle is positioned correctly in relation to the monitor's puncture line, an aspiration needle is inserted into the follicular cavity. Its content is aspirated and the follicular cavity is curetted and/or flushed several times with modified PBS to create turbulence (Pieterse et al. 1991, Brück et al. 1992).

Within the follicular cavity, the oocyte is surrounded by many layers of cumulus cells, which together form the so-called cumulus oophorus. Once detached from the follicular wall, the oocyte surrounded by cumulus cells is termed the cumulus oocyte complex (COC). Flushing the follicular cavity requires that the follicle's diameter is at least 5 mm in order to avoid the COCs being flushed backwards and forwards within the dead space of the aspiration needle; this will result in a considerable decrease in the number of cumulus cells surrounding the oocyte (Brück et al. 1997). Alternatively, a double lumen needle may be used (Bracher et al. 1993, Cook et al. 1993, Duchamp et al. 1995). Oocyte recovery rates improve with increasing needle diameter, bevel length, and vacuum pressure, but the latter also increases the proportion of denuded oocytes (Bols et al. 1997, Fry et al. 1997). Because fertilisation rates and subsequent developmental competence of denuded oocytes are reduced (Tanghe et al. 2002), a compromise in the vacuum pressure must be sought.

Recovery rates vary with species, individual animals, follicular maturity, and experience of the person performing the aspiration (Merton et al. 2003). In cattle, twice-weekly follicular aspirations at 3- to 4-day intervals prevent a dominant follicle being selected, and this has proved to be an efficient regimen for repeated follicular aspirations with a yield of two to eight COCs from each aspiration session (Merton et al. 2003, Kruip et al. 1994, Broadbent et al. 1997, Garcia & Salaheddine 1998). In mares, oocyte recovery rates from subordinate follicles are approximately 30% to 50% and 70% to 80% from preovulatory follicles after hCG treatment (reviewed by Hinrichs 2016 and Bøgh 2003).

In the early OPU studies in cattle, no hormonal stimulation was used, but subsequent studies have shown that pretreatment with FSH over 2 to 3 days, followed by follicular aspiration 1.5 to 2 days after the last FSH treatment, the so-called 'coasting', may yield a larger number of oocytes with improved developmental competence (Blondin et al. 2012). Today, in some commercial settings, often depending on practical and economical considerations, hormonal pretreatment is routinely used.

Transvaginal oocyte recovery can be performed also on pregnant cows and mares as long as the ovaries are still accessible by rectal palpation, i.e., during the first trimester, with no effect on the ongoing pregnancy (Meintjes et al. 1995a, Meintjes et al. 1995b, Goudet et al. 1998, Cochran et al. 2000, Chastant-Maillard et al. 2003).

Consequences for the Donor

It has been documented that the procedure of transvaginal follicular aspiration conducted under controlled conditions is well tolerated by the cow and mare (Petyim et al. 2000, Bøgh et al. 2003). Occasionally, follicular evacuation may result in intrafollicular haemorrhages (Brück et al. 1997, Chastant-Maillard et al. 2003, Petyim et al. 2000, Gibbons et al. 1994, Gastal et al. 1997). In addition, prolongation of the luteal phase in both cattle and mares may occasionally occur, as some aspirated follicles luteinise, especially when preovulatory follicles are evacuated (Duchamp et al. 2003, Goudet et al. 1997, Amiridis et al. 2000). Progesterone production will be lower and the life span shorter of these CL-like structures when formed from subordinate rather than from dominant follicles (Petyim et al. 2000, McEvoy et al. 2002, Bøgh et al. 2003), but the animals usually continue to cycle normally and fertility is uncompromised with subsequent normal pregnancy rates obtained (Brück et al. 1997, Pieterse et al. 1991, Kruip et al. 1994, Broadbent et al. 1997, McEvoy et al. 2002, Bøgh et al. 2003, Kanitz et al. 1995). Repeated follicular aspiration can continue over several months, but in most donors a decrease occurs at some point in the number of follicles available and thus in the oocyte yield, both in cows and mares (Duchamp et al. 1995, Merton et al. 2003, Kruip et al. 1994, Boni et al. 1997, Kanitz et al. 2000). Finally, because each aspiration sessions includes one epidural anaesthesia, repeated sessions can result in formation of more connective tissue in the epidural canal (McEvoy et al. 2006). This does not cause lasting problems for the donors, and it can be minimised by use of smaller needles, careful injection technique, and by varying the injection site.

Additional Applications of Transvaginal Follicular Puncture

The development of this rapid and relatively simple technique has also provided opportunities for other procedures, such as oocyte transfer from donor to recipient (Carnevale et al. 2000), evacuation of anovulatory follicles and follicular ablation (Brück et al. 1995), as a research tool to study follicular development and to synchronise follicular growth (Garcia & Salaheddine 1998, Brück & Greve 1996, Bergfelt & Adams 2000, Brück et al. 2000, Shaw & Good 2000, Kim et al. 2001, Bøgh et al. 2002). It has also been used as an experimental tool to inject oocytes into follicles to obtain final maturation and subsequent ovulation, followed by fertilisation after insemination and then embryo collection at Day 7 in the horse (Hinrichs & DiGiorgio 1991, Deleuze et al. 2009) and in cattle (Kassens et al. 2015).

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Transvaginal ultrasound-guided follicular ablation can also be used in conjunction with superovulatory regimens. Aspiration of all follicles of more than 5 mm (Bergfelt et al. 1997), or only the dominant follicles (Merton et al. 2003), or a combination of follicular ablation with hormonal treatments with GnRH, E2 and/or progesterone (Bo et al. 2006, Martinez et al. 1999, Amiridis et al. 2006) will lead to the emergence of a new follicular wave approximately 3 days after aspiration; at this stage the superovulatory treatment may be initiated.

In addition, oocyte transfer, also called gamete intrafallopian transfer (GIFT), may be attempted as an alternative to *in vitro* embryo production in the mare. For this purpose, *in vivo* matured oocytes may be recovered 28 to 36 hours after hCG treatment of the donor mare, or immature oocytes may be recovered for *in vitro* maturation from mares after sudden death or euthanasia. The matured oocytes are then transferred surgically via a flank incision into the oviduct of either cyclical, inseminated recipients, whose oestrous cycle has been synchronised with that of the donor and whose preovulatory follicle has been previously aspirated to remove the oocyte so that it is not available for fertilisation. Alternatively, ovariectomised or nonovulating mares, pretreated for 3 days with oestrogen and inseminated, can be used (Hinrichs et al. 2000, Carnevale et al. 2003). Oocyte transfer via catheterisation of the oviduct with transabdominal laparoscopy has been performed successfully (Kollmann et al. 2011) but requires additional equipment and training.

In vitro Embryo Production

General Principles/Purpose

In vitro embryo production is normally designated IVF, even though in domestic animals it consists of three distinct *in vitro* phases: maturation of oocytes (IVM), fertilisation of oocytes with capacitated spermatozoa (IVF), and finally culture of the presumptive zygotes (IVC). Collectively, the whole procedure is designated *in vitro* embryo production (IVP) and results in IVP embryos. Chang (1959) published the first report of the birth of rabbits after IVF, and subsequently, live births after IVF were reported in the hamster and mouse (Iwamatsu & Chang 1969). In the domestic species, Brackett et al. (1982) were the first to report the live birth of a calf after IVF, using *in vivo* matured oocytes aspirated from follicles at laparotomy, with surgical transfer of the presumptive zygotes to the oviduct of synchronised heifers after 24 to 48 hours of *in vitro* culture. The first calves born after IVM and IVF were reported by Hanada et al. (1986), and almost simultaneously Xu et al. (1987) and Lu et al. (1987) reported pregnancies and birth of IVM-IVF calves. However, Xu et al. (1987) cultured the presumptive zygotes in the oviduct of heifers (intermediate recipients), flushed them from the uterus, and then transferred an embryo to the final recipient, yielding a normal calf. Live births were also reported after IVF from other species, including lambs by IVM/IVF (Cheng et al. 1986), piglets by IVF (Cheng et al. 1986), piglets by IVM/IVF (Mattioli et al. 1989), and foals by IVM/IVF (Palmer et al. 1991, Bézard et al. 1992).

Today, IVP is most widely used in cattle, but even after 30 years of research IVP-embryos are more 'fragile' than those which are *in vivo* derived. For a review and greater details on the development of bovine IVP, culture conditions, and other aspects, see Gordon (1994) and Avery (2006). Details of the merits of *in vivo*-derived versus *in vitro*-produced embryos are described later in this chapter.

Cumulus oocyte complexes (COC) from slaughterhouse-derived ovaries are generally aspirated from follicles more than 2 mm in diameter, as they have proved to contain developmentally competent oocytes (Fair et al. 1995). Other techniques, such as dissecting individual follicles or slicing the surface of the ovaries, have also been used, but for all practical purposes the aspiration technique is preferred for almost all species. After aspiration, the COCs are classified according to the number of layers of cumulus cells and the appearance of the ooplasm, although the latter may be difficult if the oocyte is surrounded by a tight layer of cumulus cells. Because the COCs originate from follicles at various sizes and developmental stages (nonatretic and atretic), their quality will vary. Irrespective of species, oocytes without a cumulus investment (denuded oocytes) and oocytes with signs of degeneration are normally discarded before IVM. The presence of at least two layers of cumulus cells is indispensable in order to allow the bidirectional communication required for adequate maturation and subsequent fertilisation and development (Gilrichst et al. 2004).

During IVM of the COCs, most oocytes resume meiosis and reach the metaphase II stage by end of the culture period. The cumulus investment surrounding the oocyte expands, and about 80% to 90% of the oocytes will extrude the first polar body, which is round and considerably smaller than the second polar body; the latter is extruded into the perivitelline space after fertilisation. The cytoplasmic and the nuclear maturations occur simultaneously. It is the intention to mimic *in vivo* maturation as close as possible, but it is clear that, although the nuclear kinetics of the *in vivo*- and *in vitro*-matured oocytes morphologically appears to be similar, the *in vivo*-matured COC is much 'stickier' than its *in vitro* counterpart. Hyttel et al. (1997) have described the details of the oocyte maturation process. When the oocyte reaches the MII stage, IVF is initiated. Therefore the duration of the IVM phase should be adjusted according to follicular maturity to the *in vivo*-maturation period for that particular species and to culture conditions.

The spermatozoa used for fertilisation must be capacitated *in vitro*, and various media and procedures have been used for this purpose. High ionic-strength medium was initially used by Brackett et al. (1982), but a major breakthrough for cattle IVF was achieved when Parrish et al. (1989) added heparin to the media and achieved *in vitro* capacitation. A motile fraction of spermatozoa may be obtained using a variety of different methods, such as simple washing and centrifugation, swim-up techniques, density gradient centrifugation, or filtration through glass wool; laboratories vary in their preferred choice (Avery 2006, Parrish & Foote 1987).

The COCs and spermatozoa are cocultured for 6 to 24 hours, and during the very early period most cumulus cells are shed. Upon contact between spermatozoa and zona pellucida, the acrosome reaction is initiated. Then the spermatozoa penetrate the zona pellucida and usually only one spermatozoon gains access to the perivitelline space, where it may move around before fusion with the oolemma. The most efficient ratio of spermatozoa to oocytes may vary from species to species but is generally much higher than during *in vivo* fertilisation. The cortical granule reaction is somewhat different for *in vitro* versus *in vivo* fertilisation (Hyttel et al. 1987), and for certain species, such as the pig, polyspermia is a serious obstacle to success.

The *in vitro* culture period will inevitably vary with species and the purpose of IVP, and within the first 24 hours the second polar body will be extruded (Fig. 44.17). In cattle, for example, the presumptive zygotes are normally cultured for 6 to 7 days before transfer, but in experimental studies, shorter and even longer *in vitro* periods have been used.

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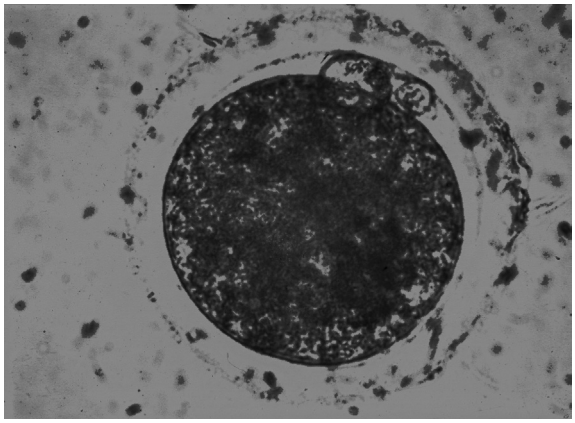
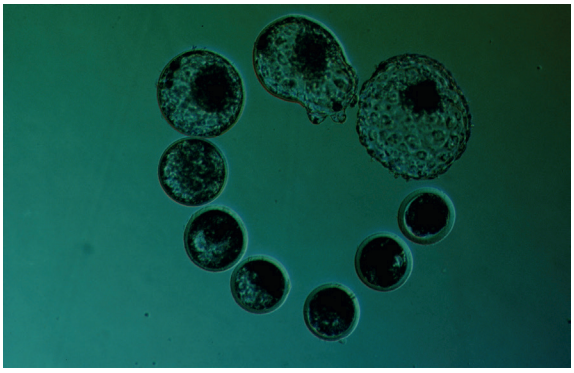


• **Fig. 44.17** *In vitro*-fertilised cattle zygote showing both first and second polar body. Note the difference in size between the two.



• **Fig. 44.18** *In vitro*-produced cattle embryos on day 7 to 8 after fertilisation.

A fundamental element in the *in vitro* procedures is the media used. The first were based on different standard cell culture media with certain modifications believed to be suitable for the various steps of the development. Because it has been and still is a major area of research to optimise and improve them, a number of different media have been and still are used for the various species, e.g., Hams F-10, TALP, CR1, Menezo B2 and NCSU, M199/Hanks; in addition, a number of commercially produced media are also available today. The medium of choice varies from species to species, and in some coculture with cells is also used. Metabolic studies of oocytes and embryos have been useful, first of all to better understand their basic nutrient requirements at different developmental stages and thus to design more physiologically acceptable media for *in vitro* culture. In the early embryo, cell division is an exponential process that each time requires the duplication of the essential cell components. Thus an efficient and adequate supply of energy substrates, precursors is required for the DNA and RNA synthesis of DNA and electron carriers. The most important energy sources for early embryos of several mammalian species are carbohydrates (Rieger et al. 1989, Brück & Hyland 1991, Brück et al. 1997).

In vitro Embryo Production in Cattle

Over the last two decades, commercial *in vitro* production of cattle embryos has increased dramatically, with more than 400,000 embryos transferred worldwide in 2015 (Perry 2016). In addition, numerous IVP embryos have been produced for research purposes. Oocytes are obtained either from slaughterhouse-derived ovaries or after OPU. Using the first source, Lu and Polge (1992) created a bank of cheap embryos from animals of average genetic merit, and this approach is still used, for example, to export embryos to countries which need a rapid increase in a higher genetic-valued cattle population. Commercially, OPU is used to obtain oocytes from elite donor heifers or cows that are inseminated with semen from elite bulls. Over the last years, this breeding system has been further accelerated by use of genomic profiling of the IVP embryos based on a biopsy from the preimplantation embryos (Ponsart et al. 2014).

From slaughterhouse-derived ovaries, an average of eight to nine oocytes per ovary can be expected, and after OPU around 15 per animal. For example, the number is dependent on the breed as several beef cattle breeds may give much higher numbers. The selected COCs are placed in groups in culture medium, and one might expect that 80% of the oocytes reach the MII stage, 80% cleave, and after IVC, 40% reach the blastocyst stage. The speed of development is different among the zygotes, which explains why different developmental stages are seen on Day 7 (Fig. 44.18). It should be emphasised that there is a very great animal-to-animal variation, not only in the number but also in the quality of the aspirated oocytes (Tamassia et al. 2003).

Sex-sorted spermatozoa have been used to produce IVP embryos and calves since the 1990s (Cran et al. 1993, Lu et al. 1999), and today it is used routinely in several commercial operations. In general, cleavage and blastocyst rates are lower than after the use of unsorted spermatozoa (Hayakawa 2012) with a considerable variation between bulls (Blondin et al. 2009).

Pregnancy rates after transfer of IVP embryos are generally 10% to 20% lower than their *in vivo* counterparts (Hasler 1998, Hasler 2006, Pontes et al. 2009). As mentioned previously for *in vivo* transfer, factors such as embryo quality and recipient management influence these rates.

ICSI has not been widely used in cattle, but embryo development (Zhang et al. 2003) and birth of live offspring has been reported (Horiuchi et al. 2002). Freezing of blastocysts after ICSI is also possible (Keskintepe & Brackett 2000).

In vitro Embryo Production in Horses

In vitro production of equine embryos was initially considered to be a promising alternative to superovulation and embryo transfer, and in 1990 two foals conceived after IVF of *in vivo*-matured oocytes were born (Palmer et al. 1991, Bézard et al. 1992). In spite of worldwide attempts, this has never been repeated, and no live foals have been produced by IVF since. Refinement of the IVF technique for the horse has progressed only slowly, which appears to be related to the inability of the sperm to penetrate the zona *in vitro*. Attempts have been made to solve this challenge, and acceptable *in vitro* fertilisation rates have been obtained by breaching the zona pellucida through partial dissection or dissolution (Choi et al. 1994, Li et al. 1995) or by pretreatment of sperm with calcium ionophore (Zhang et al. 1990, Dell'Aquilla et al. 1996, Alm et al. 2001, Hinrichs et al. 2002). The low IVF rates can be circumvented by ICSI, allowing the establishment of pregnancies and birth of live foals after transfer of early stage embryos into the oviduct of recipients (Cochran et al. 1998, Meintjes et al. 1996, Squires et al. 1996, McKinnon et al. 2000, Hinrichs et al. 2007). Extension of the postfertilisation culture period has provided the possibility of nonsurgical intrauterine transfer of blastocysts produced from

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in vitro-matured oocytes fertilised by ICSI (Li et al. 2001, Galli et al. 2002, Choi et al. 2003).

The first prerequisite for successful IVP is access to meiotically competent oocytes, optimisation of oocyte retrieval, and adjustment of IVM conditions to the different maturity stages of recovered COCs. *In vivo*-matured oocytes can only be recovered from preovulatory follicles by OPU, and because of the lack of an efficient superovulation treatment for mares, the availability of these oocytes is limited to one or two per oestrous cycle. Oocytes recovered from subordinate follicles are generally immature and have to be matured *in vitro*. Thus in an equine IVP programme, oocytes recovered *in vivo* 24 to 36 hours after hCG treatment will still have to undergo partial IVM to metaphase II, whereas immature oocytes recovered from subordinate follicles (e.g., from the ovaries of a mare after sudden death) are generally immature and will have to be matured *in vitro* for 24 to 36 hours.

Many investigations have been undertaken to improve IVM conditions, and IVM success evaluated as nuclear maturation rates varies considerably among laboratories. There is ample evidence that these variations are due to biological and methodological factors such as age of mare, follicular size and atresia, time of ovary transport, method of oocyte recovery, culture media, and culture time; generally, a 50% to 80% MII rate may be expected (reviewed by Hinrichs (2016) and Bøgh (2003)).

Because IVF as practised in cattle is not a feasible method, ICSI is currently the method of choice. Choi, Hinrichs, and their colleagues have contributed significantly to the development of ICSI in the horse. Approximately 20% to 35% of the injected oocytes develop into blastocysts, both after *in vitro* embryo culture and *in vivo* after transfer into the oviduct of a recipient mare (Choi et al. 2004, Choi et al. 2003, Hinrichs et al. 2005, Hinrichs 2016). Importantly, these rather high developmental rates were achieved with immature oocytes. ICSI with frozen–thawed sperm has been performed without jeopardising the developmental rate (Choi et al. 2006), so ICSI is a potential method for foal production from stallions with acquired subfertility or with limited reserves of frozen semen.

IVP requires optimisation of each of the individual technical steps: retrieval of oocytes and sperm, oocyte maturation, sperm capacitation, fertilisation, embryo culture, and embryo transfer. In horses the actual IVP is generally the prerogative of an experienced research team and a well-equipped research laboratory, although the veterinary practitioner participates with donor and recipient management, supply of high quality semen, and the actual embryo transfer. The clinical use of ICSI using oocytes recovered postmortem from mares of high economical value has become a speciality in which the veterinary practitioner may provide a service to the horse owner with relatively limited means. Ovaries should be excised from the mare as soon as possible after death, preferably before euthanasia but under general anaesthesia. If transport time to the laboratory performing ICSI is less than 2 hours, ovaries can be transported at body temperature (37°C); otherwise, they should be cooled down to 15° to 20°C during the transport. Oocytes derived from ovaries recovered from mares immediately before euthanasia as well as up to 5 hours postmortem have been associated with production of foals (Carnevale et al. 2003, Ribeiro et al. 2008, Hinrichs 2016).

In vitro Embryo Production in Sheep and Goats

IVP of sheep and goat embryos follows the basic principles mentioned for cattle and has been reviewed in detail by Cognié et al. (2003); minor differences are related to the method of oocyte retrieval and the IVM procedure. Obviously, conventional cattle OPU cannot be performed in small ruminants, but COCs can be obtained by laparotomy or laparoscopy-guided ovum pick-up, yielding about six COCs and one to two blastocysts per session (Tervit 1996, Kühholzer et al. 1997). Regarding IVM, addition of cysteamine to the IVM medium will increase the blastocyst yield in goats (10% vs 50%). After surgical transfer of two IVP embryos per recipient, kidding percentage and percentage of kids born per transferred embryo of approximately 60% and 50%, respectively, may be expected. ICSI has also been successfully accomplished in sheep, with birth of a male lamb (Catt et al. 1996, Gomez et al. 1998), but, as in cattle, this method has not gained widespread use.

In vitro Embryo Production in Pigs

IVP of porcine embryos has not gained widespread commercial use, and it is still primarily used for experimental purposes. After IVM, IVF, and IVC, several authors have reported birth of piglets; for review on IVP of porcine embryos, see Grupen (2014).

In comparison to IVP methods in cattle and horses, the IVM period for pigs is 40 to 45 hours. In addition, in the start of the 2000s the use of North Carolina State University (NCSU) medium supplemented with porcine follicular fluid or bovine fetal calf serum resulted in considerable higher blastocyst recovery rates than was seen with other media (Peters et al. 2001). Today, other media, such as PZM-3 (Yoshioka et al. 2002) and commercially available media, are routinely used for porcine IVP.

The selected COCs are placed in groups in culture medium, and the nuclear maturation rate reaches the same level as in cattle (80%–90%), whereas the cleavage rate approaches 40% to 50%, and approximately 20% of the fertilised oocytes can reach the blastocyst stage. These figures illustrate that one major problem is still encountered in porcine IVP, namely a high incidence of polyspermic fertilisation, which is at least 30% (Nagai et al. 2006); in addition, several chromosomal abnormalities have been documented (McCauly et al. 2003). The fertilisation process is the principal cause that is illustrated by the quite successful use of the IVM and IVC procedures, as key parts of somatic cell nuclear transfer (SCNT).

Reports on ICSI in pigs are scarce, although this technique has the advantage of avoiding the problem with polyspermia. Kolbe and Holtz (2000) achieved a cleavage rate around 25% and obtained one pregnancy after surgical transfer of two- to four-cell stage embryos, and even sex-sorted sperm may be used for this purpose (Probst & Rath 2003).

In vivo-derived versus *In vitro*-produced Embryos and Advanced Quality Measurements

General Comments

It has become increasingly clear that *in vivo*-derived and *in vitro*-produced embryos are substantially different both morphologically and functionally.

Morphologically, Avery and Greve (1995) and Van Soom (1996) have described some of the features and differences between *in vivo* and *in vitro* embryos. Compared with their *in vivo* counterparts, IVP embryos: are darker, probably because of their higher lipid content; are less compacted; the total cell number is lower; the

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zona pellucida is more fragile; the ratio between inner cell mass and trophectoderm (ICM:TE ratio) is lower; and the density is lower due to the higher lipid content. At the ultrastructural level, several differences are obvious regarding organelle distribution of for example mitochondria and cortical granules (Maddox-Hyttel et al. 2003) and certain biochemical features such as fat content and protein composition (Farin et al. 2001).

Functionally, *in vivo*-derived embryos are quite robust in response to different environmental conditions, such as temperature and light, whereas IVP embryos are more fragile and less tolerant. One very important feature is their increased sensitivity to chilling and to cryopreservation (Greve et al. 1992, Leibo & Loskutoff 1993). In contrast to *in vivo*-produced embryos, male IVP embryos develop faster than female embryos (Avery et al. 1992, Peippo et al. 2001) in some IVP systems but not in others (Holm et al. 1998, Pontes et al. 2011); therefore this characteristic cannot be used to guarantee that embryos selected at a later stage (Day 7) for transfer will result in a higher proportion of a specific sex.

All of these features are clearly a consequence of the artificial conditions under which the process is performed; thus improvement of the culture conditions (temperature, atmosphere, media) may reduce these differences. It has been unclear which one of the three steps in the *in vitro* production system may have the most significant effect on embryo quality, but IVC appeared to be the most sensitive part (Lonergan et al. 2003). Currently, the focus is mainly on the competence of the immature oocytes used at the very start of the *in vitro* process (Blondin 2017).

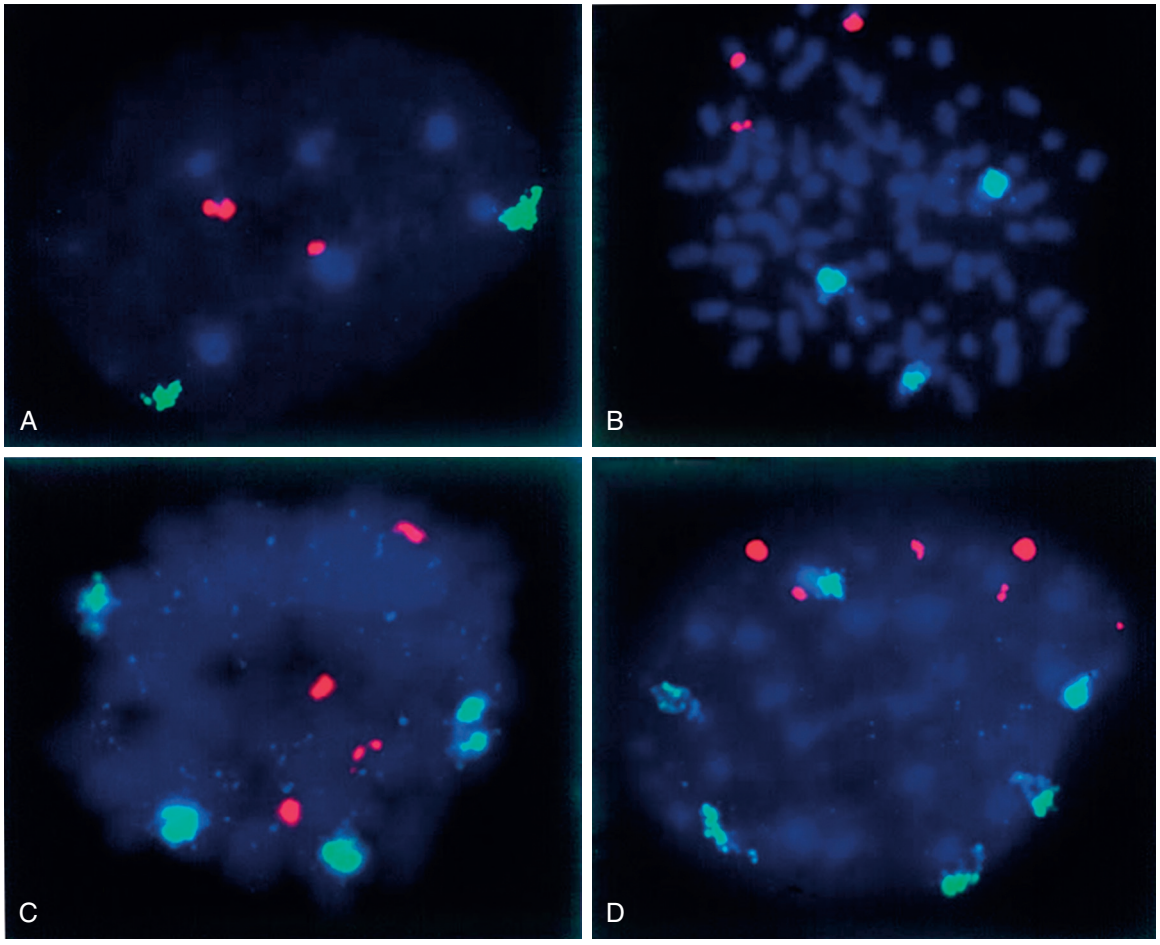
Different Measures of Embryo Quality

As a measure of embryo quality, the classical morphological method described earlier has been used because it is simple to perform and thus useful under practical conditions. However, with the differences experienced between *in vivo* and *in vitro* embryos, it has become even more important to find other and additional quality measures, as the use of morphology has proved to be of limited value in explaining and understanding how to evaluate *in vitro* embryos. Together with rapid appearance of various new technical possibilities, a range of more or less sophisticated methods for evaluating embryo quality have been tested and will be described.

When considering the methods of evaluation, it is important that the method used does not itself adversely affect embryo quality. Thus there are clear limitations on the thoroughness of the evaluation method, and hence the only realistic method for embryo evaluation in veterinary practice is by means of light stereomicroscopy.

Fluorescence In situ *Hybridisation*

Fluorescence *in situ* hybridisation studies (FISH) allow localisation of specific DNA and RNA sequences and are suitable for chromosome analysis in cells from embryos. Viuff et al. (1999) demonstrated that around 75% of Day 7 to 8 IVP bovine embryos were mixoploid, compared with only 25% of their *in vivo* counterparts (Fig. 44.19). Chromosomal anomalies may reduce the viability of IVP embryos, and chromosome analysis of a biopsy from an embryo may thus be used to assess viability. Another important difference between



• **Fig. 44.19** Fluorescence in situ hybridisation of cattle embryos indicating normal diploidy (A), triploidy (B), and other types of polyploidy (C, D). (Courtesy Dorthe Viuff, Denmark.)

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in vivo and IVP embryos is the incidence of apoptosis measured by TUNEL, in which IVP embryos have a higher apoptosis index than in vivo-derived embryos (Maddox-Hyttel et al. 2003, Gjørret et al. 2003), and it is anticipated that a higher index is inversely related to viability. The culture medium, duration of culture, and oxygen tension seems to influence the degree of apoptosis.

Frequent Microscopical Observations

Frequent stereo- and light-microscopical observations (time lapse) of oocytes and embryos can be used to assess the developmental pattern of oocytes and embryos without interfering with their normal development, and the time intervals to first and subsequent cleavages seem particularly important (Van Soom et al. 1997, Holm et al. 2002, Lonergan et al. 1999). Studies of this type are obviously tedious and time consuming, but semiautomatic time-lapse recording systems are easier to use and may be used to estimate embryonic developmental kinetics (Peippo et al. 2001, Holm et al. 2002). In the latter study, clear kinetic differences were observed between *in vivo*-derived and *in vitro*-produced embryos cultured *in vitro*, as the second, third, and fourth postfertilisation cell cycles were shorter for *in vivo*-derived embryos. The initial cleavage time is known to be correlated with embryo quality, as measured by pregnancy rate and cryotolerance of IVP embryos (Lonergan et al. 2003), and therefore kinetic studies may be useful to predict embryo viability. This knowledge is utilised in several commercial products, in which semiautomatic time-lapse recordings are an integrated part of the embryo culture system used for the selection process of the most viable embryos for subsequent transfer. Such equipment is mostly used for experimental purposes in farm animals, whereas it is more or less routine in human fertility clinics.

The development of multiphoton laser scanning microscopy, combined with specific intravital fluorophores, has been used to visualise particular structural or molecular features during *in vitro* development of mammalian oocytes and embryos (Squirrel et al. 1999, Petersen et al. 2008). It appears that these techniques may be optimised to such an extent that subsequent developmental competence may be predicted, for example, as part of the time-lapse systems.

PCR Techniques

The rapid advances in polymer chain reaction (PCR) techniques (e.g., RT-PCR and DD-RT-PCR) have had a major influence on understanding normal embryo development, e.g., activation of the embryonic genome (Viuff et al. 1998, Watson et al. 1999). Many studies have clearly shown that genes of developmental importance are expressed differently when embryos are produced *in vitro* and that these deviations may be used to evaluate the quality of a given culture system (Bertolini et al. 2002, Niemann et al. 2002, Rizos et al. 2002). It has also been documented that embryos with deviant gene expression patterns have increased sensitivity to cryopreservation as an indication of poorer quality (Lonergan et al. 2003). Chromosomal anomalies are also more pronounced in *in vitro*- than *in vivo*-developed embryos (Viuff et al. 2002).

Metabolic Studies

Metabolic studies of oocytes and embryos were developed in an attempt to evaluate embryo quality and viability (see review by Donnay (2002)), but it is also very useful as a way to monitor the development and thus can be used as an additional quality measurement (Rødgaard et al. 2015). Practically, this can be performed by sampling the culture media after their use ('spent culture medium') in order to analyse for various substances that may reflect the normality of the individual embryo's development. In some semiautomated culture systems, its use is becoming a realistic possibility, together with the use of time-lapse observations.

In 1996, Thompson et al. (1996) developed a noninvasive microfluorescent technique to measure the rate of oxygen uptake, and Overström (1996) reviewed work on studies using specific gas-sensing microoxygen electrodes for evaluating the quality of *in vivo* cattle embryos subsequently measured by pregnancy rates. Using a similar approach on single bovine embryos, Shiku et al. (2001) found a strong correlation between the embryo's quality and oxygen consumption. In the same period a microsensor technique was developed to measure partial oxygen pressure in the surrounding culture medium of individual IVP bovine embryo (Lopes et al. 2007a). Oxygen tension varied considerably among and between *in vivo*-derived and *in vitro*-produced embryos (Lopes et al. 2007b), and there was a significant correlation between oxygen tension and expression of certain developmentally important genes (Lopes et al. 2007c). There was no effect of the measurements per se on the survival rate of *in vivo*-derived embryos after nonsurgical transfer (Lopes et al. 2007b), which may render this technique a useful noninvasive tool for quality assessment.

The culture systems are extremely important for the development of IVP embryos, and domestic animal embryos seem to reach a higher developmental rate when given the possibility to grow in groups of approximately 10 to 20 (Gil et al. 2003). According to some views, it is a more natural situation to culture embryos individually compared with group cultures, and in certain cases it is necessary to culture the embryos individually in special microenvironments. This is already a routine in human IVF, and with the use of embryo biopsy for use in genomic selection, it is also needed in cattle to keep the embryos individually. For this purpose, several systems have been developed that allow for paracrine interactions between the individual embryos, such as small dents made in a four-well dish, the so-called well-of-the-well system (WOW; Vajta et al. 2000), the glass–oviduct system (GO; Thouas et al. 2003), agar compartments (Peura 2003), or a polyester mesh system (Matoba et al. 2010). The WOW system results in a consistent and high level of development into blastocysts of bovine IVP embryos (Vajta et al. 2000) and has also been successfully applied to produce cloned, zona-free embryos (Booth et al. 2001, Vajta et al. 2003). Another approach to this small-size culture system is based on microfluid technology. Beebe et al. (2002) obtained higher blastocyst development rates of murine embryos with such equipment, and the technique has also proved to be effective for porcine embryos (Clark et al. 2003, Walters et al. 2003). It may ultimately not only lead to an easier IVP method but also facilitate studies on, for instance, substrate requirements. In addition, this approach could become the basis for a more or less automated system for future *in vitro* embryo production (Le Gac & Nordhoff 2017).

Cryopreservation of Oocytes and Embryos

Purpose/General Principles

The overall purpose of cryopreservation is to ascertain that the cells can be stored for an unlimited time, at least in theory, and only if the storage temperature is sufficiently low, e.g., by using liquid nitrogen. Having accomplished this, the cryopreserved oocytes or embryos can be stored for many different purposes, e.g., embryo banking and transportation. The general cryopreservation principles are similar for all mammalian cells. Nevertheless, as embryos contain

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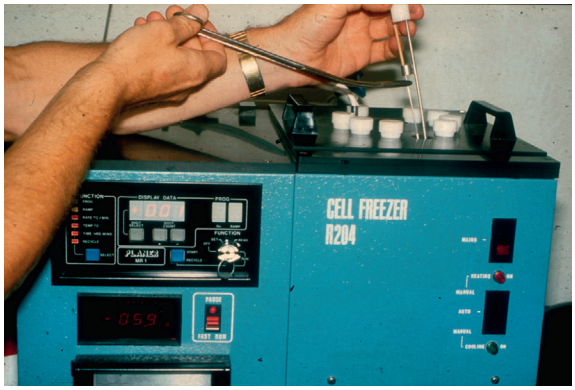

• **Fig. 44.20** Freezing of cattle embryos in an automatic cell freezer; induction of crystallisation (seeding).

a fluid blastocoele and as both oocytes and embryos are surrounded by a zona pellucida and contain considerably more cytoplasm than spermatozoa, the loading of cryoprotectants and the intracellular dehydration and rehydration processes are different compared with other cell types (Leibo 1977, Lehn-Jensen & Rall 1983).

The first successful freezing of cattle embryos was reported by Wilmut and Rowson (1973), applying the principles developed in mice by Whittingham et al. (1972). It led to the birth of the calf 'Frosty 2', but the overall success rate of the technique was low. Subsequently, the technique was refined for sheep and cattle (Willadsen 1977, Willadsen et al. 1978), and this procedure has now been applied to most other domestic species. The studies have shown that Day 6 to 7 sheep and cattle embryos at the morula, early blastocyst, or blastocyst stage are more tolerant to low temperature preservation than earlier and later developmental stages.

The main principle in this 'slow rate freezing–thawing procedure' is to use low concentrations of the intracellular cryoprotective agent, normally 1.5 mol/L glycerol or 1.5 mol/L ethylene glycol in Dulbecco's PBS supplemented with 20% FCS, or using a commercially available medium. The embryos are equilibrated for 10 to 15 minutes, then loaded into a straw and placed in the chamber of a manual or automatic freezer at −6°C. After approximately 5 minutes, ice crystal formation ('seeding') is induced with supercooled forceps (Fig. 44.20), and a slow freezing rate is used until −35°C, at which stage the embryos are plunged directly into liquid nitrogen at −196°C.

A good selection of simple freezing equipment is now available at relatively low cost and this, together with the high survival rate at least of ruminant embryos after freezing and thawing, has resulted in cryopreservation becoming a routine procedure in practice.

Exposing the straw for a short period of time to air and then submerging it in a water bath at 25° to 30°C for a few minutes is the best method of thawing, irrespective of species. When embryos are frozen/thawed in ethylene glycol, they can be transferred directly (Voekel & Hu 1992), whereas embryos frozen/thawed in glycerol must be submitted to a procedure in which the cryoprotectant is removed stepwise before transfer. Sucrose, a nonpermeating cryoprotectant, may be used to dilute the glycerol, and this seems to improve the embryo survival rates.

Obviously, injuries may occur during freezing and thawing, and these include osmotic shock, intracellular ice crystal formation, fracture damage, and chilling injuries; detailed descriptions of these have been presented by Leibo (1977) and Lehn-Jensen and Rall (1983).

An alternative to slow freezing is an 'ultrarapid freezing and warming procedure', the vitrification method that is defined as a glass-like solidification of solutions at low temperature without ice formation (Vajta et al. 1997a). Rall and Fahy (1985) and Massip et al. (1986) were the first to use this method successfully in mice and cattle embryos, respectively. It has subsequently been used to cryopreserve oocytes and embryos of most other domestic animal species, in particular porcine embryos, as well as IVP and cloned embryos, which are more sensitive to freezing than their *in vivo* counterparts (Du et al. 2007, Van Wagtendonk-de Leeuw et al. 1997).

Vitrification requires addition of high concentrations of the cryoprotectant followed by ultrarapid freezing and warming, with an inherent risk of osmotic and toxic injuries. In contrast to the slow rate of freezing/thawing, injuries by ice crystal formation and chilling are virtually nonexistent (Vajta et al. 1997a, Vajta et al. 1997b). In an improved modification of the vitrification method, Vajta et al. developed the so-called open pulled straw (OPS) vitrification method, which basically reduces the fluid volume surrounding the embryo during vitrification to a minimum (Vajta et al. 1997a).

The vitrification procedure may vary from laboratory to laboratory and from species to species, but common to all is that the vitrification protocol, in particular the equilibration time, must be followed very carefully in order to obtain satisfactory survival rates. High concentrations of cryoprotectant, as with vitrification, may result in irreversible depolymerisation of the microfilaments and microtubules (Overström et al. 1993). To use the method commercially, each embryo must be handled and the straws marked individually, so the time required for vitrification is likely to be at least as long as when using slow rate freezing. The previously mentioned vitrification methods may also pose certain biosecurity challenges as the straws are unsealed, and for most practical purposes, slow freezing is still the preferred method for preserving embryos under practical conditions. However, several methods other than the OPS are available – for example. the microdroplet (Yang & Leibo 1999), the cryoloop (Lane et al. 1999), the Cryotop method (Kuwayama 2007), or various modifications of these, whereby disease transmission in the liquid nitrogen may be prevented.

Efforts have also been directed towards oocyte cryopreservation, and it has been shown that both immature and mature equine and bovine oocytes can be vitrified in an OPS system without affecting their competence to reach metaphase II (Hurtt et al. 2000), and bovine oocytes can even tolerate a second vitrification after IVF and IVC, resulting in birth of calves (Vajta et al. 1998). Although cryopreserved and thawed *in vivo*-matured oocytes, after transfer to an inseminated recipient, can lead to the establishment of a normal pregnancy and birth of healthy offspring (Maclellan et al. 2002, Squires et al. 2003), cryopreservation of immature oocytes is less successful. Transmission electron microscopy and confocal laser scanning microscopy show that cryopreservation of immature oocytes results in cellular injury near the sites of the gap junctions between cumulus cells and the oocyte (Hochi 2003) and reduced meiotic spindle quality (Tharasanit et al. 2006). Different aspects of oocyte cryostorage have been reviewed (Ledda et al. 2007).

Cryopreservation of Cattle Embryos

Approximately 60% of all transferred *in vivo*-derived cattle embryos have been frozen (Perry 2016), mostly using the slow rate freezing method, although vitrification is also used. Extensive data are available on pregnancy rates after transfer of frozen–thawed embryos; for example, Hasler et al. (1995) showed that it is possible

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to obtain a pregnancy rate of 64% with grade 1, Day 7 embryos. Embryonic loss after transfer of frozen–thawed embryos is slightly higher than that reported for fresh embryos, and poor quality frozen–thawed embryos will even experience a slightly increased gestation length (Callesen et al. 1996).

IVP cattle embryos are, as mentioned earlier, less tolerant of chilling and cryopreservation than their *in vivo* counterparts (Greve et al. 1992, Leibo & Loskutoff 1993), and several studies have clearly shown that the pregnancy rate after transfer of IVP embryos is lower than that after the transfer of *in vivo*-derived embryos (Hasler et al. 1995). Possibly, a more fragile cytoskeleton and/or the higher lipid concentration in IVP embryos contribute to the reduced survival.

Vitrification seems to be a promising alternative to preserve IVP embryos (Donnay et al. 1998). The method was successfully applied to cattle embryos already in 1986 (Massip et al. 1986), and in a comparative study, it was found that conventional slow freezing and vitrification of *in vivo*-derived embryos yielded similar pregnancy rates (Van Wagtendonk-de Leeuw et al. 1997). For IVP embryos, Vajta et al. (1998) achieved high hatching rates when culturing 6- to 7-day-old blastocyst *in vitro*, and nonsurgical transfer of warmed OPS vitrified embryos has given pregnancy rates of about 50% to 60% (Jacobsen et al. 2003).

Cryopreservation of Horse Embryos

The first birth of a foal from a frozen embryo was reported by Yamamoto et al. (1982), followed by several others (Slade et al. 1985, Czlonkowska et al. 1985). In principle, it is possible to combine the different ARTs with cryopreservation of embryos. Pregnancies can be obtained from *in vivo*-collected, *in vitro*-matured oocytes after ICSI with frozen–thawed semen, and subsequent IVC to the blastocyst stage, which are then conventionally frozen and transferred after thawing (Hinrichs 2016). Nevertheless, developmental competence is often reduced with increasing amount of *in vitro* interference, and cryopreservation may contribute to this after exposure to cryoprotectants that alter the embryonic metabolism (Rieger et al. 1991).

Although freezing of bovine embryos has found routine application in commercial embryo transfer programmes, the cryopreservation of equine embryos has been more complex and less successful. The survival of equine blastocysts after cryopreservation is complicated by the development of the capsule, which apparently hinders the permeability and equilibration of cryoprotectants (Legrand et al. 2000). There is a clear correlation of embryonic size and survival after cryopreservation, with more than 300 µm having higher survival rates (Squires et al. 1989, Boyle et al. 1985, Hochi et al. 1995, Caracciolo di Brienza et al. 2004). Partial removal of the capsule with trypsin treatment has led to a decreased percentage of necrotic cells after cryopreservation, although there was no clear advantage in the term of pregnancy rates (Legrand et al. 2000, Legrand et al. 2002, Maclellan et al. 2002). Puncturing, followed by partial removal of the blastocoele before vitrification using micromanipulation equipment or manually with a 25-gauge needle, has been used with good results in embryos larger than 300 and up to 650 µm in diameter, even though pregnancy and foaling rates are still lower than for embryos smaller than 300 µm (Ferris et al. 2016, Choi & Hinrichs 2017).

Several cryoprotectants, including glycerol, ethylene glycol, DMSO, methanol, and 1,2-propanediol, have been tested on equine embryos (Landim-Alvarenga et al. 1993, Bruyas et al. 1997, Young et al. 1997, Bass et al. 2004), and glycerol and ethylene glycol are generally used, having resulted in birth of live foals when the conventional slow freezing procedure has been employed (Slade et al. 1985, Seidel et al. 1989, Hochi et al. 1996). Equine blastocysts exhibit a higher permeability to ethylene glycol than to glycerol (Pfaff et al. 1993), and ethylene glycol or a combination of glycerol and increasing concentrations of ethylene glycol combined with vitrification resulted in acceptable pregnancy rates (Hochi et al. 1995, Caracciolo di Brienza et al. 2004, Hochi et al. 1994, Eldridge-Panuska et al. 2005).

Thus currently, the most practical commercial approach in veterinary practice without the availability of micromanipulation equipment is to collect embryos for cryopreservation just when they enter the uterus 5.5 to 6 days after ovulation. This is before capsule development and with a maximum diameter of less than 300 µm, even though the embryo recovery rate for Day 6 embryos is significantly lower than for later stages (Squires 2016). For the slow freezing approach, the embryos are exposed at room temperature to increasing concentrations of cryoprotectant in two steps (5% v:v glycerol in DPBS for 10 minutes, 10% for 20 minutes), then loaded into 0.25 mL straws, sealed, cooled from room temperature to −6°C at 1°C/min and held at −6°C for 5 more minutes. The straws are seeded, held at −6°C for another 5 minutes, and subsequently cooled to −32°C at 0.3°C/min, then to −35°C at 0.1°C/min before being plunged into liquid nitrogen (Lascombes & Pashen 2000). This is a time consuming procedure and requires a programmable freezer. Alternatively, the embryos may be vitrified, which involves stepwise equilibration in a medium containing increasing concentrations of ethylene glycol of up to 40%, or glycerol plus ethylene glycol, loaded into 0.25 mL straws or low volume micropipette tips that allow for vitrification by direct plunging into liquid nitrogen. Pregnancy rates of up to 45% to 67% can be achieved this way (Hochi et al. 1995, Eldridge-Panuska et al. 2005, Hinrichs & Choi 2016).

The thawing procedure for equine embryos follows closely the general principles of thawing embryos described previously (Squires et al. 1989, Seidel 1996). With the previously described methods, embryos smaller than 300 µm in diameter can be frozen with reasonable success leading to pregnancy rates of 50% to 75% (Squires et al. 1989, Jacob et al. 2012, Slade et al. 1985, Choi & Hinrichs 2017, Lascombes & Pashen 2000).

Cryopreservation of Sheep and Goat Embryos

Slow rate freezing of small ruminant embryos follows the principles outlined for cattle and has been used extensively (Tervit et al. 1986). Several vitrification studies have shown that it is possible to achieve pregnancy rates of 50% to 60% after direct transfer (Baril et al. 2001, Hong et al. 2007), whereas direct laparoscopic transfer of vitrified sheep embryos (Okado et al. 2002, Isachenko et al. 2003) yields lower rates (20%).

Cryopreservation of Pig Embryos

Pig embryos at most stages of development are sensitive to cooling and freezing (Polge 1977), probably because of their high lipid content. Nevertheless, it was possible to produce offspring from slow freezing of expanded blastocysts (Hayashi et al. 1989). It seems advantageous to use perihatching stages (Kuwayama et al. 1997), and removal of the lipid droplets seems to improve the survival rates considerably (Nagashima et al. 1995, Dobrinsky et al. 1998).

However, vitrification has resulted in high *in vitro* survival rates (Kobayashi et al. 1998, Holm et al. 1999, Berthelot et al. 2000),

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and many studies have subsequently shown that vitrification is the preferred method for both *in vivo*-derived (Cameron et al. 2004, Ushijima et al. 2004), *in vitro*-produced embryos (Hiruma et al. 2006), and even cloned porcine embryos (Li et al. 2006).

Additional Embryo Manipulations

ARTs include many, often unphysiological, manipulations of the gametes, e.g., moving them from one dish or tube to another as well as placing them under quite different conditions (temperature, light, media, atmosphere). Furthermore, some procedures are considered to be a routine part of today's set of ART, such as cryopreservation. Several other manipulations are also available, but they are far more sophisticated and are primarily for experimental purposes. These manipulations are of a physical, chemical, or biological nature and will be discussed briefly here.

Bisection

In the late 1970s, Willadsen made great advances in developing techniques for micromanipulation and bisection of embryos in domestic animal species. In 1979 he produced identical (monozygotic) twins by microsurgical bisection of two-cell stage sheep embryos (Willadsen 1979). The technique involved embedding the zygotes in agar before culture in ligated sheep oviducts and subsequent surgical transfer to synchronised ewes. A few years later, the birth of twin lambs and monozygotic quadruplets, monozygotic triplets and monozygotic twin calves after microsurgical separation of four- and eight-cell stage embryos was reported (Willadsen 1981, Willadsen & Polge 1981). Willadsen et al. (1981) produced monozygotic cattle twins from embryos collected nonsurgically. In both sheep and cattle, the success rate is inversely proportional to the stage of embryo development. Thus, if 16-cell stages are used, development to the blastocyst stage is very low, and the cell number is too low to allow establishment of pregnancy.

A simpler technique to produce identical twins in cattle from morula and blastocyst stages was described later (Ozil 1983, Williams et al. 1984, Picard et al. 1986). The method involves opening the zona pellucida and cutting the embryo in two parts either: (1) inside the zona pellucida, leaving one half within the original zona and transferring the other half into a surrogate zona obtained from another oocyte; or (2) removing the embryo proper and bisecting it on the bottom of the dish outside the zona, before transferring the two halves into the original and a surrogate zona. Initially, agar embedding before transfer was used, but pregnancy rates after direct transfer without the agar procedure seem to be virtually identical (Warfield et al. 1987). Comparable pregnancy rates may be expected as for whole embryos (Bredbacka et al. 1992).

Despite an increased calving rate after transfer of bisected embryos (approximately 120%) and its possible use as a research tool whereby genetically identical individuals can be placed in different treatment groups (e.g., nutrition and environment), this technology has never been widely used. Zona pellucida is an effective barrier against infection, and opening it by microsurgery has reported to cause infection with BVD virus; this risk must be taken into consideration.

Chimerism

Experiments on interspecies sheep and goat chimerism showed that mixing blastomeres from 4- and 8-cell stage sheep and goat embryos can result in the formation of viable blastocysts that, after transfer, can give rise to viable sheep–goat chimeras (Fehilly et al. 1984). After these initial studies, several other groups have successfully produced chimeras from sheep–goat (Polzin et al. 1987), ovine (Butler et al. 1987), and cattle (Brem et al. 1984). Attempts to produce live offspring of horse–donkey chimeras have not been successful, although pregnancies were established (Pashen et al. 1987). Chimera production has no direct practical applications, but the technology may constitute an important experimental tool for studies of fetal–maternal interaction.

Biopsy

From the developing embryo, a biopsy with one or more cells can be taken for various purposes (Mullaart 2002). This can be done by using a micromanipulator with which the maximal precision can be obtained so only one blastomere can be isolated if required, but it can also be done by hand if more cells are needed and if loss of more cells can be tolerated by the embryo. This biopsy can be used for different molecular studies, either to determine the embryo's sex or to describe the complete genomic profile (Hinrichs 2016, Ponsart et al. 2014). Both applications are discussed further in a later paragraph.

Cloning

Willadsen (1981) observed that separation and culture of blastomeres from 16-cell stages could not give rise to live offspring. These findings led him to follow alternative means of producing identical individuals, and in 1986 he published his initial results with cloning of sheep embryos (Willadsen 1986). Ten years later, Campbell et al. (1996) produced live sheep offspring using a differentiated cell population of embryonic cells. Cloning with cultured cells from fetal and adult tissues, the so-called somatic cell nuclear transfer (SCNT), resulted in the birth of the sheep 'Dolly' in 1996 (Wilmut et al. 1997). The donor cell source was the mammary gland, and since then, cells from many other tissues have been used successfully, e.g., oviductal epithelial cells (Goto et al. 1999), fetal fibroblasts (Zakhartchenko et al. 1999), granulosa cells (Wells et al. 1999), and skin fibroblasts (Hill et al. 2000). Also since then, this technology has been applied successfully with reports in scientific journals of the birth of viable offspring from a large range of species representing both ruminants and many monogastric species; so far no primate offspring has been reported.

There are two major components to cloning, namely the oocyte and the donor cell. In cattle and sheep, IVM oocytes are routinely used in many laboratories. The genetic material (MII plate and the first polar body) of the mature oocyte is first removed to avoid ploidy abnormalities and arrested development (Willadsen 1986). As the MII is not readily visible using light microscopy, the polar body and part of the ooplasm anticipated to contain the metaphase plate are removed either by micromanipulation using a suction pipette or by manual bisection with a scalpel blade. The enucleated oocyte is then called a cytoplast. Alternatively, removal of the chromatin (enucleation) may take place after having stained the DNA, with subsequent confirmation of complete enucleation using a fluorescence microscope.

With traditional cloning, a so-called 'karyoplast', meaning a somatic cell derived from either early stage embryos (A), embryonic stem cell-like cells (B), cultured somatic cells from a fetus (C), or an organ (e.g., skin or udder (D)) is injected under the zona of the cytoplast, i.e., the enucleated matured oocyte (Fig. 44.21). The cell membranes are subsequently fused using a direct current

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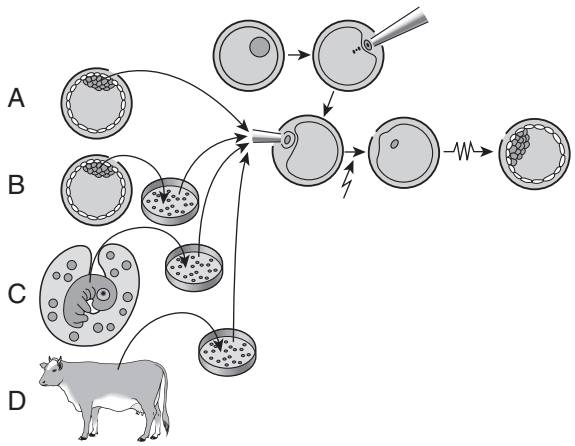

• **Fig. 44.21** Cloning by nuclear transfer using various cell types. (Courtesy Poul Hyttel, Denmark.)

• **Fig. 44.22** Surgical transfer of cloned embryos to the tip of one uterine horn in the pig.

• **Fig. 44.23** First piglets produced by handmade cloning born in 2006.

electric pulse, and the resulting zygote is activated by means of, for example, 6-DMAP or calcium ionophore. It is important that the oocyte and the donor cells are in the relevant stage of their cell cycle (G0 or G1), to achieve optimal reprogramming of the donor cell genome, i.e., that the adult cell is able to be programmed as a newly fertilised egg. In the initial experiments, the reconstituted embryo was cultured in ligated sheep oviducts, but, with the rapid advancement of IVC, this *in vitro* method is now the most commonly used when the cloned embryo is cultured; often the embryo is transferred rapidly after completion of cloning to avoid a subsequent culture step. The WOW system is very useful when culture of individual reconstituted embryos is desired, as it creates a suitable microenvironment and prevents the zona-free zygotes and embryos from disintegrating, which they might otherwise do in a normal-sized medium droplet. Comprehensive reviews of the number of offspring after different activation protocols and *in vitro* culture in different media are available (Niemann et al. 2011, Cibelli et al. 2016).

Most of the aforementioned results are based on the use of micromanipulatory equipment, but the so-called 'handmade cloning' (HMC) was subsequently developed (Booth et al. 2001, Peura et al. 1998, Vajta et al. 2001, Oback et al. 2003). No micromanipulatory equipment is needed, and the procedure differs from the classical method in two ways: (1) At 21 hours after the start of maturation (Day 0), cumulus cells and zona pellucidae are removed and oocytes randomly bisected by hand in a dish; (2) After a repeated fusion step with two fibroblasts, reconstructed embryos are activated with calcium ionophore and dimethyl aminopurine before they are cultured for 5 to 6 days (Holm et al. 1999) using the WOW system (Vajta et al. 2000). Using the HMC technique, it is possible to bisect 95% of the oocytes, to fuse 85% successfully and obtain a blastocyst rate of about 50%. In our initial studies, when Day 7 HMC cattle embryos were transferred nonsurgically, we obtained a pregnancy rate of 48%, but by the end of the third semester, only 8% were pregnant (Pedersen et al. 2004). The first HMC piglets were born in 2006 (Du et al. 2007) (see Figs 44.22 and 44.23), and since then, a rather robust system has been developed (Callesen et al. 2014) with high pregnancy rates (approximately 90% over a 4-year period) and an abortion rate around 10%.

It must be emphasised that the success rate of cloning generally is still very low and rarely exceeds a blastocyst production rate of 2% to 5% (Liu et al. 2015) and a rate of 15% piglets born per cloned embryos transferred (Callesen et al. 2014). This is due to a number of factors, such as improper epigenetic reprogramming (methylation pattern; for reviews see Farin et al. (2006) and Campbell et al. (2007)) of the somatic cell nuclei resulting in aberrant expression of developmentally important genes (Niemann et al. 2002, Wrenzycki et al. 2004) and a high incidence of chromosomal abnormalities (Slimane-Bureau & King 2002), including entirely polyploid embryos (Booth et al. 2003). Collectively, these abnormalities will not only cause poor embryonic development but can also have effects on the course of pregnancies (conceptus loss) and later also the offspring (see discussion later in this chapter). Nevertheless, the efficiency of the cloning of horses is surprisingly high, with a report of a pregnancy rate of 25% after transfer of eight cloned horse embryos (Hinrichs et al. 2006).

SCNT per se is not routinely used in livestock production due to low efficiency and high costs, but it may be used for certain very valuable animals, e.g., elite breeding bulls or show jumping horses. In several countries the technology has also been used in pets and endangered species. However, there are ethical concerns regarding these applications, and in some countries, particularly in the EU, cloning of pet animals, cattle, and horses is not allowed for production purposes. It should be added that the legislation in most countries prohibits human consumption of cloned animals, although there are no reports indicating that meat or milk from cattle, swine, or goat clones or their progeny should present a risk for human health (Kochhar & Rudenko 2010).

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Transgenesis

In 1985 the first transgenic pigs were produced by injecting copies of human growth hormone genes into the pronuclei of newly fertilised eggs (Hammer et al. 1985). The technique was subsequently used in several studies (Pursel et al. 1990), and comprehensive reviews are available (Cibelli et al. 2016, Wall 1996, Niemann & Kues 2000). However, as a consequence of the random insertion of the injected transgene, a number of problems were encountered with the offspring. Furthermore, it was not known if and how the transgene was positioned before the birth of the animal, so today this approach is not used in larger animals.

With the emergence of SCNT, it became possible to produce transgenic embryos by genetic modifications of the donor cells before fusion with the cytoplasts. One great advantage was that the successful positioning of the transgene at the anticipated place could be tested on the cells before they were used for cloning, so the animal born was known to carry the transgene as planned. This procedure was published for the first time in pigs (Lai et al. (2002), sheep (Schnieke et al. 1997), goats (Reggio et al. 2001), and cattle (Cibelli et al. 1998). Since then, many different types of such genetically modified animals have been born for a number of purposes such as: (i) for basic research; (ii) as disease models for certain important human diseases; (iii) as bioreactors, in which pharmaceuticals for treatment of human diseases may be extracted from blood, urine, or milk; and (iv) for organ transplantation from pigs to humans (xenotransplantation). Among these, the most promising seem to be the production of model animals that may develop important human diseases such as atherosclerosis (al-Mashhadi et al. 2013) or Alzheimer's (Jakobsen et al. 2016), and a recent report illustrates that the xenotransplantation aspect is still under development (Fischer et al. 2016). The bioreactor area is still being investigated, with one example being antithrombin III produced from goat's milk to be used to treat patients with inheritable antithrombin deficiencies. However, at present only a very few drugs have been licensed for clinical practice.

It should be stressed that use of transgenic animals for specific agricultural purposes such as increased disease resistance, e.g., against bacteria causing mastitis (Hyvönen et al. 2006), has yet to be implemented, as it most likely will be far too expensive and may not effectively protect animals against the specific disease. A comprehensive review of cloning and transgenic animal production has been published by Cibelli et al. (1998).

It should finally be mentioned that transgenic offspring may also be produced by artificial insemination with spermatozoa carrying the desired construct. However, the method, designated sperm-mediated gene transfer, has so far only been successfully applied in mice and pigs (Lavitrano et al. 2006).

Consequences of *In vitro* Handling of Gametes

Manipulations of the living cells that are fundamental in the creation of a new individual may obviously have inherent negative consequences. This has been the situation ever since the first experiments were made using either sperm, oocytes, or embryos. However, it caused much attention in 1991 when Willadsen presented data from a large cloning experiment in which blastomeres from non-surgically collected embryos were used as donor cells (Willadsen et al. 1991). The overall pregnancy rate was satisfactory, but late gestational losses, very large calves, and congenital malformations were seen much more frequently than after transfer of *in vivo*-derived embryos. Furthermore, in 1996 Walker reported on the birth of lambs with unusually high birth weights after IVP (Walker et al. 1996). This was considered to be a consequence, first described the so-called 'large offspring syndrome' (LOS), later expanded to 'abnormal offspring syndrome' (AOS; Farin et al. 2010). This opened up a new research area to identify and explain problems related to the handling of gametes under artificial and unphysiological conditions.

Direct Consequences

Some of the procedures have direct effects on the gametes, either resulting in their immediate death or causing damage resulting in subsequent impaired development. Examples are numerous, e.g., sperm after the sexing procedure, oocytes after exposing ovaries to suboptimal temperatures when being transporting from slaughterhouse to laboratory before IVM, or embryos after suboptimal incubator conditions related to a failure in the gas supply or temperature. Often these adverse conditions might have a cumulative effect and thus cause different forms of damage.

The consequences, although often problematic, have the advantage of being visible soon after they have occurred. Therefore immediate action can be taken and subsequent damage prevented.

Indirect Consequences

Some other forms of gamete damage are not apparent immediately, but their effects are observed later during the subsequent embryo and conceptus development. These consequences are much more problematic because their effects are first seen sometimes a long time after the damage has occurred, and it can have a cumulative effect. With the complex technical and chemical systems in use for the ARTs, this is really a serious part of the work; the LOS/AOS mentioned previously was one such very visible consequence, but there are many others.

On Embryos

Very well-known consequences of previous damages are problems with the preimplantation development – for example, low cleavage rates, delayed or arrested embryonic development, low blastocyst rates, lower cell number of blastocysts, deviating morphological features (fragmentations, dark colours), and increasing chromosome problems. Sometimes the problems are first revealed when the embryos are used for another ART, e.g., cryopreservation, when a low survival rate after thawing can be a clear sign of previous damages. Another typical time to discover the presence of damage can be after transfer of the embryos, when low pregnancy rates or a higher rate of early abortions can be a sign.

On Fetuses

The developing fetus may experience slower development or early or late abortion, perhaps resulting from suboptimal placental formation due to chromosomal abnormalities in the trophectodermal cells. Some of these features have been revealed in cloned pregnancies resulting in retarded growth rates and enlarged placentomes (Pedersen et al. 2004, Chavatte-Palmer et al. 2006, Bertolini et al. 2002; Fig. 44.24). In polytocous species such as the pig, the problems may appear as variations in litter size and in disparity in size of different fetuses, sometimes first identified at birth.

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• **Fig. 44.24** Large placentomes in a cloned pregnancy on gestation Day 252.

On the Newborn

Emphasis on the LOS was probably because it was so apparent because of the extreme sizes of the offspring. However, Farin et al. (2010) later described the phenomenon as 'abnormal offspring syndrome' (AOS), making it clear that several other abnormalities were identified in these offspring, particularly in calves and lambs. LOS/AOS has seldom been observed in pigs and goats and has not been reported in cloned horses, although the total number of cloned foals is so low that it is difficult to assess whether and to what extent these phenomena occur in this species. The problems were considered to be associated with the high concentration of serum used in the media, and because new media preparations with much lower serum concentrations began to be used, the frequency of this problem occurring has gradually diminished.

However, some problems still exist and present clinical challenges for both the recipient and the offspring. For the recipient this can be: prolonged gestation (Van Wagtendonk-de Leeuw 2006), weak labour and stillbirth (Schmidt et al. 1996), dystocia, and need for an obstetrical intervention (Hill et al. 1999). For the calf itself, problems other than increased fetal body mass (Bertolini et al. 2002, Walker et al. 1996, Schmidt et al. 1996, Jacobsen et al. 2000) are enlarged umbilicus, hydrallantois (Hasler 1998, Wells et al. 1999, Hill et al. 1999, Pace et al. 2002), lethargy and acidosis, poor suckling reflex (Hill et al. 1999, Pace et al. 2002, Garry et al. 1996), congenital malformations (Van Soom 1996, Pedersen et al. 2004, Vajta et al. 1998, Hill et al. 1999), biochemical abnormalities, immune dysfunction, and anaemia (Sangild et al. 2000, Chavatte-Palmer et al. 2013). Many of these abnormalities will be apparent to the practitioner such as the need for assistance (close observation of recipients at parturition and possible Caesarean operation) and neonatal care of the offspring such as fluid replacement therapy.

In pigs, several reports have been made in relation to piglets born after cloning (Chavatte-Palmer et al. 2013). The general picture is similar to that described for cattle, with an additional feature that there is often a significant variation between the sizes of piglets in a litter; not all piglets are affected the same way or to the same extent. Systematic autopsies on a larger material (Schmidt et al. 2015) have revealed that all the abnormalities can occur after natural breeding but at a higher frequency and with varying occurrences between piglets of the same litter.

After the Perinatal Period

It is important to emphasise that the problems identified affect only a proportion of all offspring; most calves and piglets are born without AOS (Chavatte-Palmer et al. 2013). Those appearing normal, or surviving a problematic perinatal period, survive without further problems, and when they reproduce, their offspring are normal and are not different from their noncloned progeny. There have been some reports of sudden deaths among cloned calves at 1 year old, but the specific cause has not been determined. Other observations regarding production and reproductive characteristics of cloned versus noncloned offspring have identified differences in the timing of the onset of puberty, which according to some studies may be delayed in IVP and cloned animals (Pace et al. 2002, Jacobsen et al. 2003). In addition, follicular development may be altered, with more small follicles and fewer medium-sized and large follicles present in cloned heifers (Pace et al. 2002). For pigs born after cloning, similar types of abnormalities have been reported after several generations in individuals that have been born from the cloned founder animals.

Sex Determination and Genotyping of Embryos

In animal breeding, there is a strong wish to plan the timing of the birth of offspring to best match the breeding plan. In addition, planning also includes two other activities before mating: one is planned matings with selection of both maternal and paternal genes, and the other is use of sexed semen.

Sex Determination

Sex determination of embryos before transfer can have several advantages in cattle. Using the bisection technique, in which one demi-embryo (morula or blastocyst) is sexed and the other transferred to heifers, it was possible to obtain satisfactory pregnancy rates, even after transfer of frozen–thawed demi-embryos (Picard et al. 1985). Other groups have attempted to sex embryos by means of determining H-Y antigens, but this method has not been applied in practical breeding programmes. Determination of an embryo's sex, using PCR of Y-specific sequences, has been used in practice for a number of years, based on an embryo biopsy taken either by 'hand-cutting' or by using micromanipulation at the morula stage (e.g., Hinrichs 2016, Herr & Reed 1991, Bredbacka et al. 1995, Thibier & Nibart 1995, Hasler et al. 2002).

The use of sex-sorted sperm may replace the need for PCR sexing of embryos but, with the current low pregnancy rate using sexed sperm (see Chapter 43), particularly in cows, there is still a need for sexing of embryos (Lacaze et al. 1997).

After transfer, sexing of 2-week-old bovine embryos is possible by means of cytogenetic analysis of an embryo biopsy (Hare et al. 1976). Later in pregnancy, ultrasound scanning of the sex of the fetus is a routine in many commercial operations; it is typically made from Day 60 to 90 of gestation and with a very high accuracy (> 95%; Stroud 2005).

Genotyping

In animal breeding and production, there is great interest in using information about single genes for specific heritable diseases but also more and more for multiple genes coding for various production and health traits (Ponsart et al. 2014). Mapping of the cattle and

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pig genome has made it possible to use genotyping for certain traits in both cattle and pig production. This is done routinely on the offspring, but especially in cattle, it is now also done on embryo biopsies (Ponsart et al. 2014). Such an activity requires advanced facilities, a strong logistical set-up, and considerable experience to perform the embryo biopsies with sufficient accuracy and efficiency first to obtain a biopsy size with sufficient DNA to identify all the genes of interest, and second not to compromise subsequent embryonic development (Blondin 2017). However, this has become an integrated part of more and more breeding companies, in which OPU and IVP is being performed.

Biosecurity Precautions

Many studies have clearly shown that the risk of disease transmission by embryos is lower than with live animals, providing the embryos are treated according to guidelines set by IETS (Stringfellow & Givens 2010) and as recommended by the Office International des Epizooties (OIE) in Paris (Appendix 3.3.1 of Terrestrial Animal Health code, available online at www.oie.int).

Comprehensive reviews (Stringfellow et al. 2004, Bielanski 2007, Givens et al. 2007) have summarised the essential precautions needed to avoid transmission of important diseases, including both *in vivo*-derived, and *in vitro*-produced embryos. Most studies involving *in vitro*-produced embryos (with the inherent risk of contamination with infectious agents and subsequent washing, and transfer) and *in vivo*-derived embryos (with the inherent risk of recovering embryos from infected animals, washing, and transfer) have shown that, with proper washing (10 times) including trypsin treatment, certain infectious agents such as infectious bovine rhinotracheitis and Aujeszky's disease viruses can be removed. Embryos collected from a persistently infected animal are shown to carry a large amount of BVD virus particles attached to the zona. Transfer of these embryos after washing according to IETS standards resulted in BVD free calves (Bak et al. 1992).

With IVP embryos, the situation is different. Many oocytes are obtained from slaughterhouse ovaries and could be contaminated with various infectious agents. In addition, the zona pellucida of *in vitro*-produced embryos allows more intimate contact with the infectious agents and thus makes it more difficult to remove the microorganism from the embryo. Furthermore, there are risks of contamination during the different IVP procedures. Therefore it is necessary not only to wash the embryos 10 times but also to set biosecurity standards for the abattoir and the laboratories, including the media used for culture. Addition of certain antimicrobial agents may also be advisable.

Obviously there will always be a risk of disease transmission with embryo transfer, and thus it is mandatory that *in vitro* and *in vivo* experiments are conducted to ascertain that a particular disease cannot be transmitted using this technique. Using the IETS and OIE standards, the impression is that movement of livestock at the 'embryo stage' is preferable to doing so in the 'free living' stage not only for economic and biosecurity reasons but certainly also for the benefit of animal welfare.

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