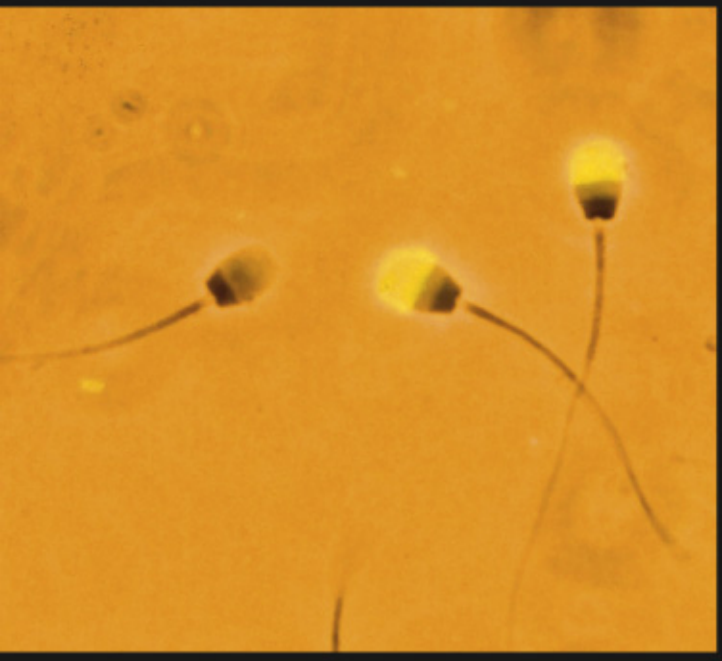
DOG BREEDING, WHELPING AND PUPPY CARE

GARY ENGLAND





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Dog Breeding, Whelping and Puppy Care

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Contents

Preface iv

Section 1: Breeding, Reproduction and Care of the Normal Animal **1**
1 Care of the Juvenile, Genetics and Planning of Breeding 3
2 Anatomy of the Female 19
3 Physiology of the Female 28
4 Endocrinology of the Female 35
5 Clinical Examination of the Female 41
6 Anatomy of the Male 64
7 Physiology of the Male 69
8 Endocrinology of the Male 74
9 Normal Mating 77
10 Clinical Examination of the Male 83
11 Fertilization and the Optimal Breeding Time 109
12 Pregnancy and Lactation 120
13 Pregnancy Diagnosis 128
14 Normal Whelping 137
15 Care of Neonatal Puppies 148

Section 2: Abnormalities of Reproduction and the Perinatal Period **155**
16 Abnormalities of the Female 157
17 Abnormalities of the Male 178
18 Intersex Animals 205
19 Abnormal Female Physiology and Endocrinology 209
20 Abnormal Male Physiology and Endocrinology 218
21 Abnormal Pregnancy 223
22 Abnormal Parturition and Problems After Whelping 232
23 Care of the Sick Neonate 246
24 Approach to Infertility Cases 254

Section 3: Control of Reproduction and Reproductive Technologies **265**
25 Hormonal Contraception 267
26 Surgical Neutering 279
27 Pharmacological Control of Reproduction 287
28 Reproductive Biotechnology 315

Index 329

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Preface

My endeavour throughout the writing of this book has been to provide a clear and practical guide that is clinically useful for the breeder, veterinary student and veterinary surgeon. I have tried to maintain an easy-to-read style with illustrations that are relevant and explain the subject area and aid understanding of normal and abnormal reproduction as well as common reproductive techniques.

Much of my clinical and research work over the last 25 years has been in the field of canine reproduction and this book represents my view of the subject area. At the suggestion of colleagues the text is written as a monograph and is referenced using my own peer-reviewed publications which demonstrate the impact of my contribution to the field. I hope that my interest and explanations will inspire further study in this exciting discipline.

The work involved in this book is a tribute to the late Dr W.E. Allen, my teacher and friend.

Gary C.W. England

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Section 1

Breeding, Reproduction and Care of the Normal Animal

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1 Care of the Juvenile, Genetics and Planning of Breeding

Puppies are most commonly weaned from their dam at approximately 6 weeks of age and will then be placed into a new home.

- the exact time of homing will depend upon the opinion of the breeder and the availability of a new home; early placement at 6 weeks has many advantages relating to behavioural development of the pup, but attention needs to be paid to health care and preventative treatments
- whilst pups may arrive in a new home 'fully weaned', there are substantial physiological changes in the gastrointestinal tract which are not complete until several months of age; these put the pup at risk of gastrointestinal upset following minor dietary change

1.1 BEHAVIOURAL DEVELOPMENT

- there are several important phases of behavioural development, including (1) the neonatal period, (2) the transitional period, and (3) the socialization period (Figure 1.1)
- on arrival at a new home, pups will have passed through the neonatal and transitional periods (these are discussed later in 15.2) and will be within the socialization period which is probably one of the most important phases of its life

Socialization period

- this period classically starts at 3–4 weeks of age and is complete at approximately 12 weeks
- during this time pups should be learning to live as part of the pack (or household)
- the period starts with a general acceptance by the pup that anything experienced is not harmful, therefore the pup will not be frightened by exposure to

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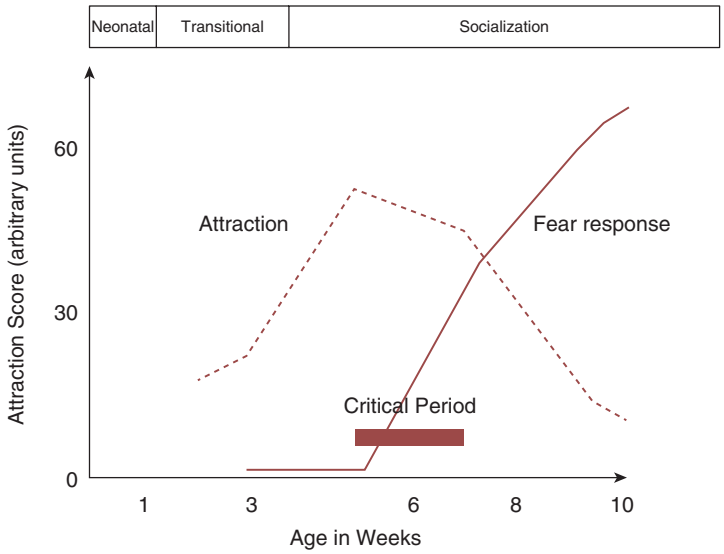


Figure 1.1 Schematic representation of attraction and fear scores for pups demonstrating the 'critical period' in relation to the neonatal, transitional and socialization periods.

something new; in the wild exposure to new things at an early age would be regulated by the dam and therefore can be considered safe

- the period ends with the pup responding to new stimuli with a fear response; in the wild as the pup roams from the nest it is more likely to meet hostile situations and these things should first be treated as dangerous
- as the pup moves through the socialization period it starts to learn what is safe, what is dangerous and how to interact with others in the social hierarchy
- a 'critical period' between 5 and 7 weeks of age has been identified and this is the time when the pup should be exposed to as many stimuli as possible so that the pup will accept these as normal and will not be fearful of them
- for appropriate socialization of the pup into the human household it is imperative that the new owner recognizes the importance of the socialization period and provides a rich, stimulating and social environment:
  (a) early exposure of pups to new environments, people, noises, stimuli etc will be accepted as non-threatening and will normally result in a life-long benefit. As examples pups who have their ears cleaned and examined at 4 weeks of age are likely to accept this as a normal event; pups that are exposed to a vacuum cleaner will accept it as not threatening
  (b) late exposure will result in a fear response that may take multiple exposures in a very controlled environment to overcome. As examples, pups exposed to a vacuum cleaner at 14 weeks of age may be fearful and it may take many months to overcome the fear

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- for appropriate socialization owners should:
  (a) make sure that pups are used to being touched all over including opening the mouth and ears
  (b) establish that it is normal for a human to remove the pups' food
  (c) establish basic training commands
  (d) expose the pups to all manner of different environments (cars, buses, shopping centres, crowds etc)
  (e) expose the pups to as many different noises as possible (fireworks, vacuum cleaners, washing machines, gunshots etc); specific tape recordings are useful for this task
  (f) ensure the pup is exposed to people of different ages, sexes and to those wearing different types of clothing, uniforms (e.g. postmen) and hats
  (g) expose the pup in a controlled environment to other animal species (e.g. cats, sheep) so that a chase response does not develop
  (h) correct all inappropriate behaviours quickly

1.2 HEALTH CARE

There is substantial alignment of the socialization period with the immune system of the pup; early in the socialization period the pup has acceptance of new stimuli (at this time the pup will have protective immunity from the dam's colostrum) whilst later new stimuli are met with a fear response (maternally derived antibodies will be waning and the pup may not be protected).

- a variety of routine preventative health care measures should be implemented and due consideration should be given to dietary requirements

Dietary requirements

- it initially appears complex to understand the dietary requirements of a developing pup, however much research has been undertaken and the use of commercially available preparations is scientifically sensible and safe in terms of nutrient requirement and bacterial contamination (many home-prepared diets do not meet the same standards for nutrients or bacteriological safety)
- the nutrient requirement will be based upon the size of the pup, the likely size at maturity (the expected growth rate) and the stage of development
- the volume of food to be fed relates to the density of the food and its nutritional value
- owners normally feed to an expected volume of food in the feeding bowl and forget that total weight of food fed is important
- overfeeding of pups is common and may result in increased tendency to obesity throughout life because of setting an expectation of a particular appetite as well as the nature of fatty tissue that is developed

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- the balance between energy intake, protein and calcium is important and will differ between breeds and body size of the dog and varies at the different stages of development; it is important either to recognize the variable amounts required if feeding home-prepared diets or to use commercially available preparations appropriate for the stage of development (age) of the pup
- it is important to recognize that commercially available complete diets for pups should never be supplemented with additional minerals as this will result in dietary imbalance and can result in skeletal deformity

Vaccinations

- vaccination regimes are designed to stimulate the body to produce an immune reaction to particular organisms
- pups will have received some antibodies from their dam (these are called maternally derived antibodies)
- maternal antibodies are not permanent but generally persist in the pup until 6 or 8 weeks of age but this varies from one infectious disease to another
- if vaccination is performed when there are high levels of maternal antibodies it is possible that vaccination will not be as successful as expected and therefore the timing of vaccination is important
- vaccinations are normally given for distemper, infectious hepatitis, parvovirus, parainfluenza, leptospirosis and Bordetella
- most primary vaccination courses include two doses at approximately 10 and 12 weeks of age although an early vaccination may be given at 6 weeks in some cases
- a later last vaccine dose may be required for canine parvovirus (e.g. at 14 or 16 weeks) because maternal derived antibody persists in the pups
- local veterinary surgeons are best placed to advise on suitable regimes since the prevalence of disease varies from one location to another

Deworming

- many breeders will have either treated a bitch for endoparasites during pregnancy, and/or treated the pups when they were in the nest
- establishing what regimes have been followed can be important and this will influence which preventative treatments are given during puppy development
- treatment is required for many different forms of internal parasites

Roundworms

- Toxocara is a roundworm that is most commonly seen in young pups and may cause respiratory disease (because of larvae passing through the lungs) or gastrointestinal disease

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- if bitches have been infected before pregnancy larvae may remain dormant but become re-activated in the last third of pregnancy and then pass to the pups; pups are born infected and may produce many eggs themselves within a few weeks of birth
- Toxocara is a potential zoonotic disease and regular de-worming is therefore imperative and should be combined with careful hygiene measures to ensure that human contamination with faecal material does not occur
- pups should be treated approximately every 4 weeks according to the instruction for the medicine supplied

Hookworms and tapeworms

- Uncinaria and Ancyclostoma are hookworms which are not uncommon in hunt and greyhound kennels
- Taenia are tapeworms, of which the most commonly seen has the flea as an intermediate host; pups with fleas invariably harbour tapeworm and should be appropriately treated
- common treatments are given at the same time as treatment for roundworm

Heartworm

- Dirofilaria is common in warmer climates since it is transmitted by mosquito
- where the condition is endemic regular prophylactic treatment is required

1.3 PHYSIOLOGICAL DEVELOPMENT

Body size

- pups grow at a rapid rate over the first 10 months of life; small and medium-sized dogs will reach adult body size at approximately 11 months of age, whereas giant breeds may not reach adult bodyweight until 20 months of age (Figure 1.2)
- there is substantial increase in bone mass and increasing length of the long bones
- normal bone and joint development can be adversely impacted by inadequate diet (both composition and volume fed)
- the period of growth is limited ultimately by the closure of bone growth plates, but there is considerable variation between these for different bones and locations (for example proximal humeral epiphysis closes at an average of 375 days whilst the medial and lateral humeral condyles close at an average of 187 days)

Body conformation

- juvenile pups normally appear relatively long-legged in appearance and this is normal

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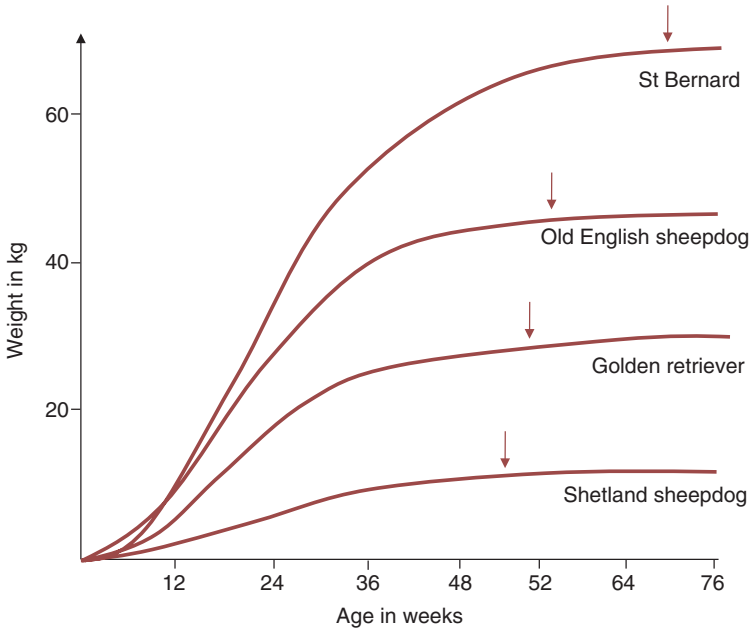


Figure 1.2 Representation of typical growth curves for a variety of different breeds of dog (arrow demonstrates achievement of adult bodyweight).

- unfortunately there has been a societal drift into acceptance of 'chunky' puppies being normal whereas 'chunky' puppies are actually overweight or obese
- accurate evaluation of pups' body conformation is best performed using an assessment of body condition score
- body condition scoring requires palpation of the dog and examining its outline, either a 5- or a 9-point scale are used for simplicity. Here a 5-point scale is described:

Score 1 **Emaciated**

Ribs, spine and bony protrusions are easily seen.

These animals have little muscle mass and there is no observable body fat.

May be described as bony, and starved in appearance.

Score 2 **Thin**

Ribs, spine and other bones are easily felt.

These animals have an obvious waist when viewed from above and have an abdominal tuck.

May be described as lean or skinny in appearance.

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Score 3 **Normal**
Ribs and spine are easily felt but not necessarily seen.
These animals have a waist when viewed from above and the abdomen is raised and not sagging when viewed from the side.
May be described as ideal and often have a muscular appearance.

Score 4 **Overweight**
Ribs and spine are hard to feel or count underneath fat deposits.
These animals have no waist which is distended or pear-shaped when viewed from above and the abdomen sags when seen from the side.
There are usually fat deposits on the hips, base of tail and chest.
May be described as heavy or stout.

Score 5 **Obese**
Large fat deposits over the chest, back, tail base and hindquarters.
These animals have a sagging abdomen and there is no waist when viewed from above.
The chest and abdomen often appear distended or swollen.
May be described as rounded or bloated.

Physical appearance and sexual behaviour

- there are no significant differences between male and female pups in their appearance at an early age
- as puberty approaches in the male the production of male hormones (testosterone) results in some specific changes including increased body size, heavier appearance of bones, lengthening of the penis and enlargement of the sheath, substantial increase in size of the testes and epididymides, increased dominance play and mounting behaviour
- in females several months before the first (pubertal) oestrus there are elevated concentrations of female hormones (oestrogens) which result in development and enlargement of the vulva (which previously may have been small and recessed) and slightly increased size of the mammary glands and teats

1.4 ONSET OF PUBERTY

Puberty is a term used to describe the transition (physiologically, morphologically, behaviourally and functionally) from a juvenile to an adult.

- usually growth of the dog or bitch has reached a plateau and changes to the physical appearance mentioned above commence and progress
- the onset of puberty is thought to be produced primarily by a change in the hormonal output from a part of the brain called the hypothalamus; in the

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juvenile period there is little activity within the hypothalamus (earlier in growth it was active), but this juvenile quiescence is followed by pulses of gonadotrophin-releasing hormone (GnRH) being produced
- the onset of puberty appears to be linked to attaining a critical body weight and to maturation of nervous tissue within the hypothalamus
- the GnRH is conducted to the anterior pituitary gland where is stimulates the release of two further hormones termed follicle-stimulating hormone (FSH) and luteinizing hormone (LH) which stimulate development of the testes and ovaries
- the gonadotrophins (FSH and LH) are the same in the male and female although it would appear strange terminology to have a follicle-stimulating hormone in a male dog which has no follicles to stimulate
- stimulation of the testis results in the production of male hormone testosterone
- stimulation of the ovaries results in the production of the female hormone oestrogen
- testosterone and oestrogen are called reproductive sex steroids
- the reproductive sex steroids are important in regulating sexual function but they also have important effects on other tissues which result in changes in their appearance and function
- the effects of the reproductive sex steroids are therefore said to influence secondary sexual characteristics; in females this results in changes in size of the genitalia and mammary glands as noted above

Puberty in the bitch (see also 3.2)

- normally reached approximately 2–3 months after achieving adult bodyweight; between 6 and 24 months of age
- small breeds reach puberty earlier than large breeds
- influenced by diet, genetic/line factors and cross-breeding
- often the bitch demonstrates some temperamental changes in the few weeks before the first oestrus including more being restless or excitable and often an increased urination (sometimes involving urine marking with one hind leg raised)
- it is important to consider appropriate housing and management to ensure that the bitch is held in a secure manner and does not escape

Puberty in the dog

- testes are normally present within the scrotum at birth
- spermatogonal activity is first detected at 5–6 months of age and first ejaculations can be collected at approximately 8 months of age
- a longer transition to adult sexual performance is noted rather than the abrupt occurrence of pubertal oestrus as in the bitch and mature semen quality is often not present until 12–15 months of age

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- dogs become more boisterous as they approach puberty and there is increased size of the testes but no other overt clinical signs

1.5 SELECTION OF ANIMALS FOR BREEDING

- as dogs mature many will be considered as potential breeding stock, and decisions are often made in relation to the appearance of the animal or the particular attributes that can be observed in him or her; this is described as the 'phenotype'
- it is important to recognize that the phenotype is a combined effect of the genes that an animal carries (the 'genotype') and the effect of environmental conditions
- selection of an animal for breeding therefore requires consideration of the qualities of the animal itself as well as its parents and grandparents and its brothers and sisters (siblings)
- overall the aim of any breeder is to produce pups that are sound in terms of temperament and health; the challenge is to select for a particular trait or suite of phenotypes without development of unwanted traits as a consequence of selection
- nowadays there are many sources of information available to help with selection of animals for breeding including specific health scoring schemes (such as hip, eye, elbow schemes) and temperamental tests (such as response of the animals to particular challenge circumstances)
- for some characteristics statistical predictors of the relative genetic value of an individual within a programme or breed are available; these are called estimated breeding values (EBVs)
- EBVs are based upon genetic liability and do not account for environmental influences
- guidance relating to the particular phenotypes for individual breeds are published as the Breed Standards and EBVs can be useful for estimating the impact that a particular mating might have in terms of shifting the phenotype in any particular direction
- for some breeds where there are especial problems guidance notes may be published (including International Breeding Guidelines) to help control specific diseases
- currently much attention is paid to selection of animals based upon health criteria and much more attention needs to be paid to selecting for temperamental soundness
- in future more information will become available about the specific gene regions that are associated with the desirable or undesirable criteria that breeders wish to select for or against; this will enable development of genomic breeding values (geBVs)
- geBVs will therefore be available for animals at a very early age and as such could be useful for selecting animals into a breeding programme before particular phenotypes are demonstrated

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- interestingly, in our laboratory we have documented heritability values for semen quality in dogs and proposed that it is possible that breeding selection may improve semen quality [1]; no other studies have investigated this in dogs and it is important to consider heritability of fertility when planning a breeding programme

1.6 BASIC GENETICS

- dogs have 78 chromosomes grouped in 39 pairs (39 from the father are paired with 39 from the mother – these are called homologous chromosomes)
- all cells contain these 78 chromosomes except sperm and oocytes where the number is halved (they contain only one set and not a pair of chromosomes)
- when sperm and eggs combine together at fertilization the chromosomes reform into pairs so the developing embryo contains 39 pairs
- chromosomes carry genes which are made of deoxyribonucleic acid (DNA)
- each gene is always found within the same chromosome and in the same place (locus) within that chromosome
- coding within each gene is governed by the order in which nucleotide bases are present; these bases are abbreviated to A,C,G,T (adenine, cytosine, guanine, thymine)
- since chromosomes are paired there will be two genes – one on each chromosome
- genes can be either dominant or recessive and the different forms of the gene are called different alleles; when describing these forms it is convention to denote dominant the dominant allele with a capital letter and the recessive with a lower case letter
- a dog with either a single or both alleles that are dominant will display that phenotype whereas for the recessive phenotype to be displayed both alleles need to be recessive
- when the two alleles are the same it is described as a homozygous state, whereas when the alleles differ it is described as a heterozygous state

Example of dominants and recessives

- consider a simple dominant recessive relationship which is coat colour in some breeds of dog (note in some breeds a more complex mode of inheritance occurs for coat colour and this is described later)
- two alleles are present black and brown; black is dominant (termed 'B') and brown is recessive (termed 'b')
- the possible combinations for an individual dog are BB, Bb or bb, where
  (a) BB = black phenotype (homozygous dominant)
  (b) Bb = black phenotype (heterozygous)
  (c) bb = brown phenotype (homozygous recessive)
- the outcome from mating depends on which alleles are present in the germ cells (sperm or eggs); an animal that is homozygous dominant (BB) can only have B alleles in germ cells, an animal that is heterozygous (Bb) will have some germ cells that are B and others that are b

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- mating (a) homozygous dominant to homozygous dominant and (b) homozygous dominant to heterozygous

	Black (BB)	
	B	B
Black (BB) B	BB = Black	BB = Black
B	BB = Black	BB = Black

(a) 100% offspring are homozygous black

	Black (BB)	
	B	B
Black (Bb) B	BB = Black	BB = Black
b	Bb = Black	Bb = Black

(b) 50% offspring are homozygous black
50% offspring are heterozygous black

- mating (a) heterozygous to heterozygous and (b) homozygous recessive to homozygous recessive

	Black (Bb)	
	B	b
Black (bb) B	Bb = Black	Bb = Black
b	Bb = Black	bb = Brown

(a) 25% offspring are homozygous black
50% offspring are heterozygous black
25% offspring are homozygous brown

	Brown (bb)	
	b	b
Brown (bb) b	bb = Brown	bb = Brown
b	bb = Brown	bb = Brown

(b) 100% offspring are homozygous brown

- mating (a) homozygous dominant to homozygous recessive and (b) heterozygous to homozygous recessive

	Black (BB)	
	B	B
Brown (bb) b	Bb = Black	Bb = Black
b	Bb = Black	Bb = Black

(a) 100% offspring are heterozygous black

	Black (Bb)	
	B	b
Brown (bb) b	Bb = Black	bb = Brown
b	Bb = Black	bb = Brown

(b) 50% offspring are heterozygous black
50% offspring are homozygous brown

Polymorphism

- some genes have more than two types of alleles
- an example are the genes that cause the spotting of colour in the coat in some breeds; here four different alleles exist but of course an individual can only have two of the four. The versions are
  - (a) S = solid colour
  - (b) s^i = Irish spotting
  - (c) s^p = Piebald spotting
  - (d) s^w = Extreme white

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Epistasis

- some genes are influenced by other genes that are present at another position in the DNA
- the gene that influences the other is called an epistatic gene
- this is nicely illustrated by the control of coat colour in Labradors where either black, brown (called chocolate) or yellow colours occur (the yellow dogs may have a dark- or a light-coloured nose)
- colour is determined by the black (B) or brown (b) allele as previously described, but also by a further allele that can be present in dominant or recessive form ('E' or 'e' respectively)
- the phenotypes and genotypes can be seen below:

Coat colour phenotype	Possible genotype
Black	BBEE BbEE BBEe BbEe
Chocolate	bbEE bbEe
Yellow (black nose)	BBee Bbee
Yellow (liver nose)	bbee

- the only genotype that can be identified from the phenotype is yellow with a liver nose that can only be bbee
- it can be seen that something as simple as mating together two black dogs is actually genetically very complicated since a black dog can be any of BBEE, BbEE, BBEe, BbEe
- one example of this would be mating a BbEe stud to a BbEe bitch:

		Stud = BbEe			
		BE	Be	bE	be
Brood = BbEe	BE	BBEE Black	BBEe Black	BbEE Black	BbEe Black
	Be	BBEe Black	BBee Yellow (black nose)	BbEe Black	Bbee Yellow (black nose)
	bE	BbEE Black	BbEe Black	bbEE Chocolate	bbEe Chocolate
	be	BbEe Black	Bbee Yellow (black nose)	bbEe Chocolate	bbee Yellow (liver nose)

56% offpring are black (with various genotypes)
25% offspring are yellow (of these 75% have black noses and 25% liver noses)
19% offspring are chocolate (with various genotypes)

Sex determination

- male and female dogs both have 39 pairs of chromosomes but there are differences in the appearance of one of these pairs between the sexes

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- in females all pairs are similar in size; but in males, in one pair, one chromosome is larger than the other – the large one is the same size as seen in the same pair in females
- these chromosome pairs are called the sex chromosomes and the larger one is designated 'X' and the smaller one is designated 'Y'; in this manner females are XX and males are XY
- when germ cells are produced by a bitch she can only have X chromosomes in her eggs, whilst for the dog he will have sperm that contain either an X or a Y chromosome
- the sex of the fertilized egg will then depend upon which type of sperm fertilizes the egg:

Female (XX)	Male (XY) X	Male (XY) Y
X	XX = Female	XY = Male
X	XX = Female	XY = Male

50% offspring will be male (XY)
50% offspring will be female (XX)

Sex linkage

- some genes are carried on the sex chromosomes; in fact very few are carried on the Y chromosome but some are carried on the X chromosome, including for example those that are responsible for the disease haemophilia
- the haemophilia gene is present in two alleles; the normal allele is dominant (H) whilst the other abnormal allele is recessive (h)
- in the female (who has 2 X chromosomes) the full gamut of normal (X^HX^H), carrier (X^HX^h) and affected (X^hX^h) will be seen
- in the male (who has XY chromomes) there is only normal (X^HY) and affected (X^hY)
- results of breeding of affected and carriers can be estimated as before, for example mating (a) affected male with normal female and (b) affected male with carrier female

Female (X^HX^H)	Male (X^hY) X^h	Male (X^hY) Y
X^H	X^HX^h = Carrier Female	X^HY = Normal Male
X^H	X^HX^h = Carrier Female	X^HY = Normal Male

(a) 50% offspring are normal males
50% offspring are carrier females

Female (X^HX^h)	Male (X^hY) X^h	Male (X^hY) Y
X^H	X^HX^h = Carrier Female	X^HY = Normal Male
X^h	X^hX^h = Haem Female	X^hY = Haem Male

(b) 25% offspring are normal male
25% offspring are carrier females
25% offspring are affected males
25% offspring are affected females

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1.7 BREEDING SCHEMES

- breeders who wish to develop or exclude particular characteristics need to understand the mode of inheritance of the condition; this may be an autosomal recessive trait or a polygenic inheritance as previously described
- for autosomal recessive traits it is imperative to understand whether an animal is normal, carrier or affected; this may be established (a) with information from the sire, dam, siblings and offspring especially by observing which litters contain affected animals, or (b) from genetic tests (for example a blood sample can be taken and gene mapping undertaken to establish if the dog is normal, carrier or affected with progressive retinal atrophy)
- for polygenetic inheritance it is possible to calculate the heritability of the trait which is the degree of variation that is caused by the genetic makeup of the animal; using this it is possible to estimate how much a population can be shifted by breeding from animals above or below a threshold of the phenotype (Figure 1.3)
- in some cases there are clear numerical data assigned to the condition that is to be selected for or against (e.g. hip score, elbow score, some trainability scores), in others the breeder subjectively assesses appearance of the individual; the aim is to breed for the wanted characteristics and against the defects
- commonly this involves breeding animals that are to some degree related (inbreeding and line breeding) but can also involve selection of animals that have appropriate characteristics from another pedigree (outcrossing)

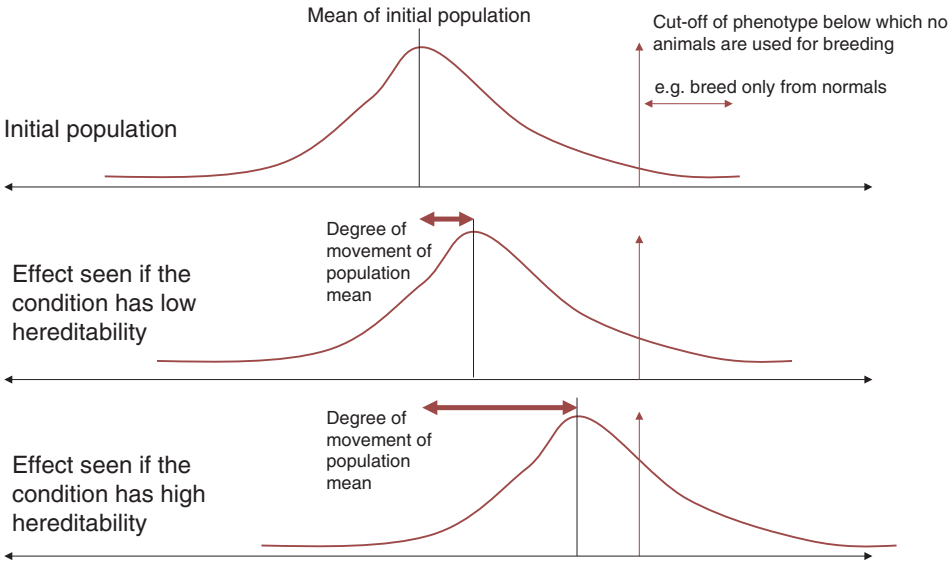


Figure 1.3 Shifting of population mean by selecting normal animals where the condition has either low or high heritability.

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Inbreeding and line breeding

- mating two genetically related animals tends to result in increased homozygosity in the programme and this is used to produce specific desired characteristics
- in fact within a country most breeds of dog have a relatively small gene pool and most animals have some degree of relation to each other
- the differences between inbreeding and line breeding relate to how closely related are animals that are bred together
- strictly speaking inbreeding is defined as breeding animals that are more closely related than the average of the population; however inbreeding it often taken to mean breeding animals that are very closely related (e.g. parent to offspring, or two siblings), whereas line breeding is taken to mean breeding less closely related animals (e.g. first cousin mating)
- the degree of inbreeding in a pedigree can be calculated using the 'inbreeding coefficient' (measured on a scale of 0–1); mating two different breeds of dog together would have an inbreeding coefficient of 0, whilst mating a brother to sister for many generations would finally result in an inbreeding coefficient of 1
- breeding from animals that have some relatives in common can be used to select for a particular phenotype, generally has rapid results (within a few generations) and can help to establish a phenotype, however it also risks establishing the unwanted genes and is essentially a genetic 'dead-end'
- there is some data to show inbreeding is associated with production of increased stillbirths and decreased litter size

Outcrossing

- here animals that do not have shared ancestors are mated
- the aim is to introduce a particular characteristic that is present in one line to the other line
- the breeder needs to be clear that within the offspring, whilst some animals will have the desired phenotype, this is likely to be a small proportion and the remainder of animals are not suitable for future breeding
- the selected animal whilst having the appropriate phenotype will in general have a heterozygous genotype and may not have the effect within a breeding programme that would be expected, or would be seen from a traditional animal that comes from a typical line-breeding programme

1.8 FIT TO BREED

- finally the potential stud dog and potential brood bitch have been selected; each must have passed the threshold criteria for health and temperament established by the breeder

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- performance of a breeding soundness examination can be useful to assess whether the individual is likely to have normal fertility (see Chapters 5 and 10); in the dog this should include collection of semen and full semen evaluation
- for the bitch there is also a question of whether she is fit to breed; in particular this decision is based upon the physical maturity of the bitch, her temperamental maturity, and her current state of health and body condition score
- nutritional factors influencing fertility are often overlooked but inadequate nutrition in both the male and female can have a significant impact

1.9 BREEDING AT THE FIRST (PUBERTAL) OESTRUS

- in a particular breeding programme there may be a desire to breed a bitch at the first oestrus so that the interval to the next generation is as short as possible
- the bitch's reproductive tract is clearly capable of sustaining a pregnancy at the first oestrus

However

- physiologically the bitch is not fully mature at the first oestrus (growth plate closure for the entire skeleton is not completed)
- many bitches are not behaviourally mature at the first oestrus
- some hereditary diseases have a late onset and may not be detectable by the time of the first oestrus
- there is a lower pregnancy rate and litter size when breeding at the first oestrus
- there is a slightly increased risk of problems at whelping when bitches are bred at the first oestrus
- overall it might be argued that breeding at the pubertal oestrus should be avoided, however, some breeds reach puberty at a much later age than others, and some individual bitches reach puberty at an age greater than the average – in these, at least, breeding at the first oestrus is entirely reasonable

REFERENCE

1 England, G.C.W., Phillips, L. and Freeman, S.L. (2010) Heritability of semen characteristics in dogs. *Theriogenology* **15**, 1136–1140.

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2 Anatomy of the Female

Understanding of the normal anatomy of the female reproductive tract is important to facilitate examination of the female for normality and to determine changes from normal which may result in disease, as well as to be helpful for establishing the stage of the oestrous cycle.

2.1 VULVA

- the opening to the female genital tract, bordered by the labia (often incorrectly referred to as the 'vagina')
- situated ventral to the floor of the pelvis (the normal position may be altered in animals with ambiguous genitalia, for example intersex)
- derived embryologically from the urogenital sinus
- size depends on: (a) breed, (b) stage of oestrous cycle (Figure 2.1)
- assessment of vulval size and tone can be useful for determining the fertile period specifically because there is swelling of the vulva in proestrus and then a significant decrease in size that occurs approximately 2 days before ovulation (see later)

2.2 VESTIBULE

- a short tube that connects the vulval opening to the vagina
- is a potential tube in that it is normally collapsed
- on the ventral wall, cranial to the ventral vulval commissure, is the clitoris which is suspended in a transverse fold of mucosa
- the fossa surrounding the clitoris is a blind-ending cavity and the inexperienced may consider it to be the opening to the vestibule which it is not
- the vestibular lumen runs cranially upwards at 60 degrees from the horizontal, and then a short distance forward in the pelvis to its junction with the vagina
- the mucosa of the vestibule is smooth and without ridges and is a pink colour

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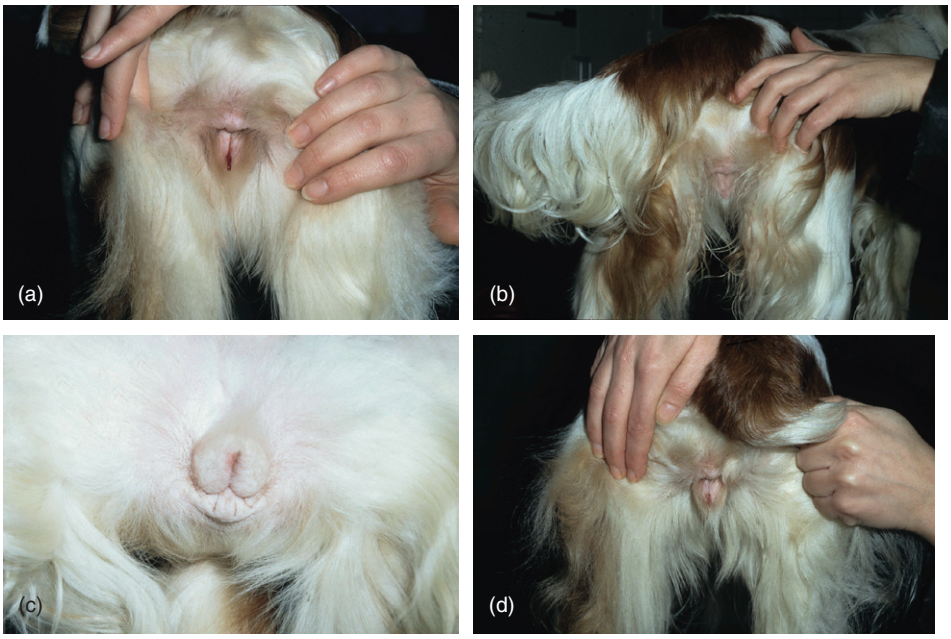


Figure 2.1 Vulval changes during the oestrus cycle: (a) pro-oestrus, note the sero-sanguinous discharge at the vulva and on the hairs of the back leg; (b) oestrus, the tail is held to one side in response to pressure applied to the back or perivulval area; (c) oestrus, the vulva is swollen and discharge is still present; (d) early metoestrus, swelling is reduced but in this bitch the haemorrhagic discharge has persisted – this is not abnormal.

2.3 VESTIBULO-VAGINAL JUNCTION

- the junction between the vestibule and the vagina
- in maiden bitches there is a constriction which indicates the position of the hymenal vestige
- in some bitches remnants of the hymen (commonly a dorso-ventral band which divides the vagina) are present and these may prevent mating or result in pain at mating
- in some bitches the hymen is totally or partially intact, thus preventing mating (see 16.3)
- the urethra opens just caudal to the vestibulo-vaginal junction on the ventral floor

2.4 VAGINA

- directed cranially from the vestibulo-vaginal junction to the cervix (level with 4th or 5th lumbar vertebra) (Figure 2.2)

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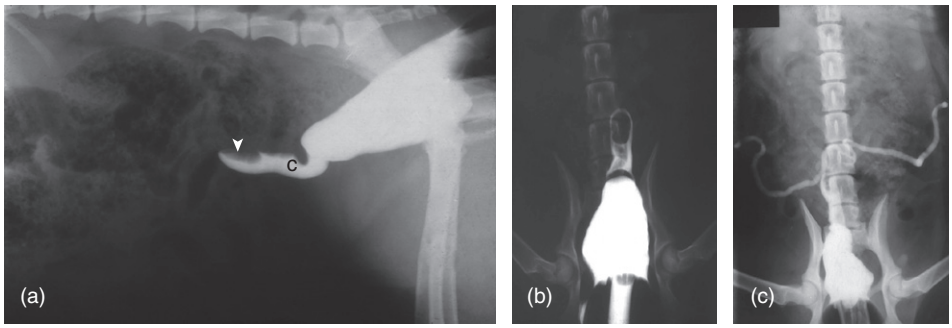


Figure 2.2 Contrast radiography of the vagina and uterus: (a) lateral view of the vagina filled with contrast medium in a bitch in anoestrus, the cranial portion of the vagina (C) is narrower than the caudal part and the arrow points to the area where the cervix joins the vagina; (b) ventro-dorsal view of the vagina of the birch in (a), note the length of the vagina. Distally there is incomplete filling of the vagina due to the cuff of a Foley catheter; (c) ventro-dorsal view of the vagina and uterus filled with contrast medium in a bitch in oestrus. (Reproduced with kind permission from the *Journal of Small Animal Practice*.)

- a potential tube lined by a stratified epithelium with longitudinally orientated folds
- immediately cranial to the external urethra orifice is a small 'tongue-shaped' ridge of mucosa; this is often large when the bitch is in oestrus (this is often the site where vaginal hyperplasia is first detected)
- cranially the vagina becomes narrow due to a dorsal median longitudinal fold of mucosa; the caudal end of this fold is often mistaken for the cervix (see 5.7)
- approximately 0.5–1.5 cm caudal to the cranial limit of the vagina, on its dorsal surface, is the opening (os) of the cervix
- the cranial two-thirds of the vagina is derived embryologically from the paired Mullerian ducts
- the epithelium changes dramatically in relation to the endocrine environment both in terms of colour, thickness of the mucosal folds and moistness
- hypertrophy of the vaginal wall occurs during oestrus
- assessment of the degree of vaginal wall hypertrophy and epithelial proliferation can be useful for determining the fertile period and therefore the optimal time for mating (see 11.3)

2.5 CERVIX UTERI

- a short, thick-walled structure with a narrow lumen which connects the vagina to the uterus
- the cervical lumen is slightly tortuous
- the vaginal opening (os) opens dorsally into the cranial vagina
- the cervical canal is directed cranio-dorsally making catheterization of the cervix very difficult

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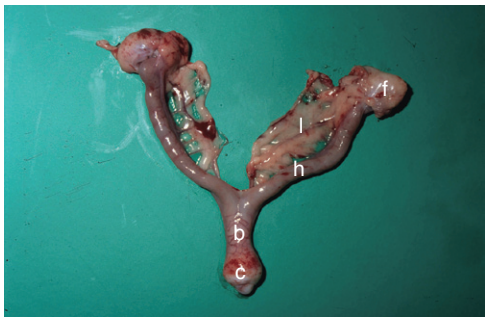


Figure 2.3 Uterus and ovaries from a bitch in early pro-oestrus showing uterine horns (h), body (b) and cervix (c). The broad ligaments (l) are continuous with the mesovarium which contains and surrounds the ovary (f).

- ventral to the cervix is a blind-ending pouch of vagina
- the cervix enlarges during oestrus and can often be palpated transabdominally
- the cervical canal is patent during late pro-oestrus, oestrus, parturition and the post-partum period; at other times it is usually closed
- there is increased secretion of mucus from the cervix and vagina in bitches in pro-oestrus and oestrus; this can be clinically useful for determining the optimal time to breed [1] (see 11.3)

2.6 UTERUS

- this is Y-shaped and composed of a short body (corpus) and two long horns (cornua) (Figure 2.3)
- the body is connected to the cervix caudally and the tips of the horns communicate with the uterine (Fallopian) tubes cranially
- the length and width of the uterus is affected by both age and physiological and pathological (see 16.5) changes
- the uterine horns lengthen and begin to form folds during oestrus
- normal changes in the non-pregnant uterus are governed by circulating hormones, principally oestrogen and progesterone (see Chapter 4)

Pre-puberty

- before puberty the uterus is small in diameter and the vascular supply is similarly small (one reason why pre-pubertal neutering is preferred by many surgeons)
- examination of the cross-section of the uterine lumen shows that this has a simple 'star-like' appearance and the endometrial glands are simple and poorly developed

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- frequently there is only very sparse fat accumulation adjacent to the uterus within the broad ligament (see 2.9)

Pro-oestrus

- at the onset of the first pro-oestrus the uterine vessels increase in size
- the endometrium becomes thicker and more oedematous and the uterine lumen takes on a complex appearance when examined in cross-section
- ultrasonographic appearance of the uterus is hypoechoic because of the endometrial oedema
- the endometrial glands become more tortuous
- extravasation of erythrocytes occurs into the lumen of the uterus, the serosanguinous discharge passes via the cervix into the vagina and characterizes the coloured discharge noted with early receptivity

Oestrus

- overall there is substantial increase in diameter of the uterus
- the uterine vessels reach maximum size
- there is less endometrial oedema and a reduction in the hypoechoic appearance
- uterine glands become more numerous and tortuous
- extravasation of erythrocytes continues into the uterine lumen so that the coloured vulval discharge continues into this stage of the cycle

Metoestrus (dioestrus)

- grossly the uterus has a 'twisted' (corkscrew) appearance associated with glandular proliferation but reduced oedema
- glandular proliferation continues until approximately the 30th day after ovulation
- in late metoestrus the glandular layer becomes more simple but often contains cystic dilations; the uterus is no longer twisted; polymorphonuclear neutrophils appear in the mucosa and necrotic epithelial tissue is seen in the lumen
- the terminology 'metoestrus' vs 'dioestrus' is discussed in 3.5 (see Figure 3.1)

Anoestrus

- the largely inactive phase of the reproductive cycle
- the uterus has a similar appearance to pre-puberty but is not as small in diameter
- in some bitches where there has been cystic dilation of uterine glands, this feature may persist into anoestrus

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2.7 UTERINE (FALLOPIAN) TUBES

- the uterine tubes run from the tip of the uterine horn to the ovary
- the tubes run within the mesosalpinx and ovarian bursa taking a meandering path which almost completely circumnavigates the ovary
- considered to have a narrow isthmus at the uterine end and a wider ampulla (where fertilization occurs) at the ovarian end; these regions cannot be grossly differentiated
- terminate in fimbriae which are attached to the openings of the ovarian bursae – the fimbriae channel the ovulated ova into the uterine tube

2.8 OVARIES

- housed within the ovarian bursa and therefore not normally visible without opening of the bursa
- ovoid in shape when inactive (anoestrus) and attached to the inner surface of the ovarian bursa
- covered by a germinal epithelium, so that ovulation may occur anywhere on the surface of the ovary (Figure 2.4)
- the size of the ovary depends upon the stage of the oestrous cycle

Pre-puberty

- peripherally within the substance of the ovary there is a layer of primordial follicles; these are small and not individually identifiable
- follicles may contain more than one ovum, although at ovulation this is rare

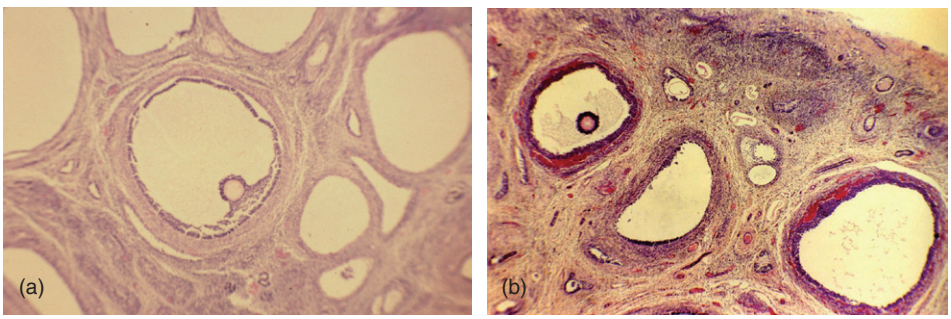


Figure 2.4 Histology of the ovary: (a) a follicle in pro-oestrus containing an ovum, the granulosa cell layer surrounding the follicle is relatively thin; (b) a follicle in early oestrus, the wall has luteinized and is producing progesterone but as yet this follicle has not ovulated.

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Pro-oestrus

- follicles enlarge due to increasing size of their fluid-filled antra
- overall the size of the ovary increases
- the internal granulosa layer of each follicle is thrown into distinct folds
- plasma oestrogen concentrations increase from late anoestrus throughout pro-oestrus

Oestrus

- a relatively small number of large follicles (4–8 depending upon breed) are identifiable on each ovary [2]
- the number of follicles is similar on each ovary and the exact number depends upon the breed and age of the bitch
- the internal layer of granulosa cells luteinize before ovulation
- this luteal tissue produces progesterone before ovulation (endocrinologically the bitch has elevated progesterone concentrations when she is still in oestrus)
- ovulation usually occurs in early oestrus
- ova are released from follicles which do not collapse but remain filled with fluid, presumably follicular fluid and blood
- luteal tissue continues to proliferate and results in gradual thickening of the follicle/corpus luteal wall

Metoestrus (dioestrus)

- the ovary is at its largest size
- the early corpus luteum does not collapse and is centrally cavitated and contains fluid
- progressive luteinization continues from ovulation onwards and occurs from the wall inwards
- by approximately 20 days after ovulation the cavity of the corpus luteum is completely replaced by luteal cells
- after this time the corpora lutea gradually become smaller and the ovary is nodular in outline

Anoestrus

- remnants of corpora lutea remain for several months and can be seen upon gross inspection
- the significance of these luteal remnants is as yet uncertain
- ovaries are larger than before puberty but generally are small in size and have a flat surface

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- in late anoestrus a new wave of follicular growth commences (often 45 days before the onset of pro-oestrus)

2.9 MESENTERY

The supporting structures of the reproductive tract which attaches the reproductive tract to the body wall, mainly dorsally.

- the terminology of the various parts of this system is governed by their position and anatomical modifications:
  (a) suspensory ligament: runs cranially from the ovary to the dorsal abdominal wall, level with the last rib; it is a strong fibrous structure
  (b) mesovarium: bounded by the suspensory ligament cranially and continuous with the mesometrium caudally; composed of loose connective tissue containing fat and the ovarian vessels
  (c) ovarian bursa: a pouch of the mesovarium which completely covers the ovary except for a small opening to the abdominal cavity; the fimbriae of the Fallopian tube are attached to this opening
  (d) mesosalpinx: a fold of mesentery running between ovary and the uterus, containing the uterine tube proper ligament of the ovary: a band of fibrous tissue which runs between the ovary and tip of the uterine horn, parallel to the mesosalpinx
  (e) mesometrium: continuous with the mesovarium cranially; loose connective tissue containing the uterine vessels

2.10 BLOOD SUPPLY

- only the vessels encountered in routine surgical procedures are described:
  (a) ovarian artery and vein: arise from aorta and empty into the vena cava dorsal to the ovary (on the left the vein empties into the left renal vein); they do not communicate with uterine vessels
  (b) uterine artery and vein: arise from the pudendal vessels near the vagina; they run cranially, parallel to each uterine horn to which they send regular branches

2.11 MAMMARY GLAND

- the number of mammary glands is variable but there are commonly five pairs positioned along the ventral thorax and ventral abdomen
- glands are designated according to location: thoracic, abdominal, inguinal, or numbered from cranial to caudal (the first gland being the most cranial one)
- glandular tissue forms the basis of the gland and glandular regions are held together with a fibrous capsule

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- contraction of muscle cells (myoepithelial cells) causes milk to be expelled into the collecting duct system
- milk passes to the outside via teat orifices; there are between 7 and 14 teat orifices per gland
- mammary glands are small during anoestrus, pro-oestrus and oestrus
- approximately 35 days after ovulation there is obvious mammary gland development
- this development occurs in all bitches, even those without clinical signs of false pregnancy
- at this time milk production is common
- mammary gland enlargement is caused by progesterone following oestrogen priming
- milk production may be seen in both pregnant and non-pregnant bitches and is associated with increased concentrations of the plasma hormone prolactin
- the blood supply to the mammary glands is important when considering surgical removal of mammary tumours; equally important is the lymphatic drainage which governs which glands need to be removed when neoplasia has been diagnosed
- the cranial mammary glands (M1 and M2) are supplied via the cranial thoracic artery; the mid gland (M3) is supplied by the cranial epigastric artery; the caudal glands (M4 and M5) are supplied by the caudal epigastric and the external pudendal artery
- there is lymphatic communication between glands M1 and M2 with drainage occurring cranially to the axillary lymph node
- there is lymphatic communication between glands M3 and M4, with M4 and M5 draining caudally to the superficial inguinal lymph node

REFERENCES

1 England, G.C.W. and Allen, W.E. (1989) Crystallization patterns in anterior vaginal fluid from bitches in oestrus. *Journal of Reproduction and Fertility* **86**, 335–339.

2 England, G.C.W., Russo, M. and Freeman, S.L. (2009) Follicular dynamics, ovulation and conception rates in bitches. *Reproduction in Domestic Animals* **44**, 53–58.

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3 Physiology of the Female

Understanding the normal cyclicity and the terminology for the stages of the cycle is a key aspect to understanding reproduction in the bitch. This is particularly the case because the physiology in the bitch is very different to that of other species. Misunderstanding of the normal physiology is one of the most common causes of infertility in this species.

3.1 TERMINOLOGY

- the terminology that is commonly used is often confusing due to historical mistakes, comparisons with other species and a tendency to 'borrow' inappropriate terminology from other species
- some terms are more acceptable because of traditional usage rather than scientific accuracy (Figure 3.1)

3.2 PRE-PUBERTY

The period between birth and the beginning of the first pro-oestrus.

- puberty can occur between 6 and 24 months or more and is substantially dependent upon the breed; with small-sized breeds reaching puberty earlier than large breeds
- throughout pre-puberty the bitch has no swelling or enlargement of the mammary glands or vulva and there is no normal discharge from these structures
- behaviourally the bitch is playful and immature
- commonly the coat retains a soft and fine appearance like that seen in puppies, the bitch is often lean and not well muscled

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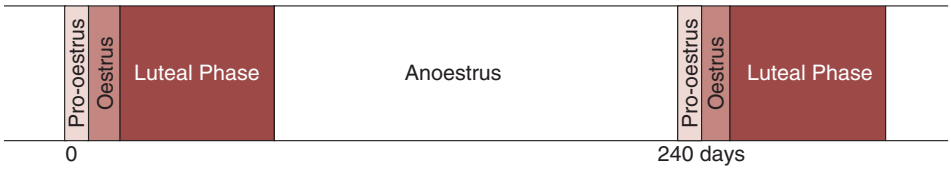


Figure 3.1 Schematic representation of the various phases of the oestrous cycle of the bitch.

3.3 PRO-OESTRUS

- lasts on average for 9 days but is very variable in duration
- often the first pro-oestrus is longer than expected and can be thought to be abnormal
- initial signs are swelling of the vulval lips and the appearance of a sero-sanguinous (red-coloured) discharge from the vulva
- the quantity of discharge varies greatly and is of no clinical significance
- the discharge may not be seen in some bitches as they remove it by licking
- urination becomes more frequent (small quantities) to disseminate pheromones produced by the vestibular mucosa
- commonly bitches play with males and may seek their attention
- the bitch is attractive to male dogs but will not usually allow coitus
- ovulation may occur towards the end of this period but normally occurs during oestrus

3.4 OESTRUS

- lasts on average 9 days but is very variable in length
- by definition this phase commences when bitch will first readily allow coitus
- by definition oestrus ends when bitch subsequently refuses to allow coitus
- the discharge from the vulva may become less copious and less haemorrhagic but this is not a constant feature, i.e. some bitches have scant discharge throughout pro-oestrus and oestrus, others have a copious 'bloody' discharge which persists after the end of oestrus
- ovulation usually occurs towards the beginning of oestrus
- in the author's laboratory we have shown for the first time the large variation in the endocrine events occurring in normal bitches; ovulation may occur within a few days of the onset of pro-oestrus or may take many weeks [1]
- it appears that the length of time that follicles take to develop is very variable (Figure 3.2); the following may happen:
  (a) ovulation may occur during a normal length pro-oestrus (as early as 5 days after the beginning)
  (b) ovulation may occur as early as 7 days after the onset of pro-oestrus and the bitch may be in oestrus at this time

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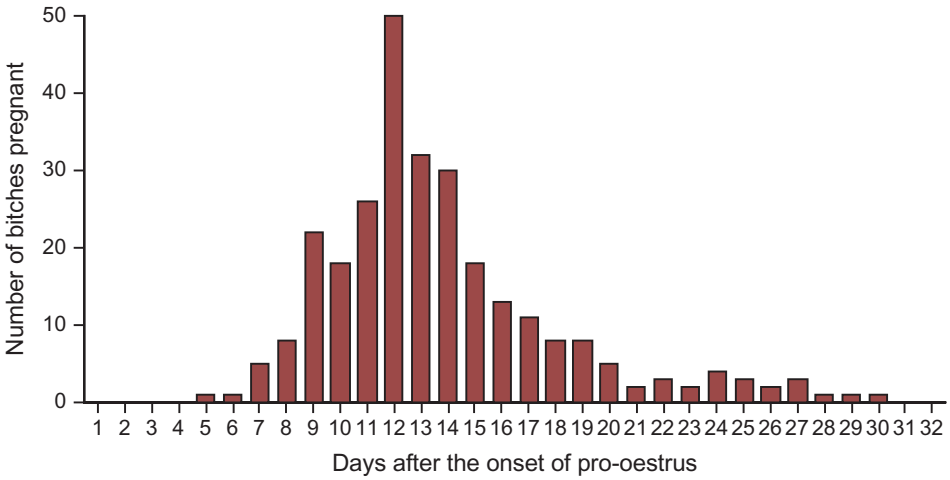


Figure 3.2 Relationship between day of ovulation and onset of pro-oestrus for 280 bitches.

(c) ovulation may occur as late as 30 days after the onset of pro-oestrus; the bitch is still receptive at this time
(d) ovulation may occur as late as 30 days after the onset of pro-oestrus; the bitch is not receptive at this time

- in addition, the time of ovulation may not be consistent during successive heats in the same bitch
- in our clinic we have undertaken some pivotal studies investigating growth and ovulation of follicles [2]; these showed a surprising long period of follicle recruitment compared with other species
- use of ultrasound to study follicular dynamics will become an important diagnostic tool following demonstration of abnormal cycles in our work [2]
- bitches release (ovulate) several eggs (oocytes) at each cycle; ovulations do not occur at the same time and this process usually takes approximately 72 hours
- most ovulations occur 48–72 hours after the plasma peak of luteinizing hormone
- because dog spermatozoa can live for up to 7 days in the bitch, and because oocytes are not ready for fertilization until 2 days after ovulation, bitches which are mated by more than one dog can have mixed litters
- in cases of paternity disputes genetic 'finger printing' is almost 100% accurate
- the duration of time that eggs (oocytes) are available to be fertilized (called the 'fertilization period') is very long in the dog compared with other species; this can result in a large difference in the time that whelping commences when pregnancy length is calculated from the day of mating (see also 11.2)

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3.5 METOESTRUS (DIOESTRUS) [3]

- in many species metoestrus is used to describe the early part of the luteal phase (often for approximately 5 days after ovulation) whereas dioestrus is the remaining non-pregnant luteal phase (often approximately 10 days)
- in the bitch the term metoestrus is traditionally used to describe the majority of the luteal phase after the end of oestrus; there is little logical reason for this, and the word dioestrus is more accurate
- in the bitch metoestrus begins when the bitch first refuses coitus
- overall metoestrus lasts about 60 days and terminates when circulating progesterone concentrations become minimal; there is no clinical sign which heralds the end of metoestrus
- after the end of oestrus the vulva gradually becomes less swollen and any haemorrhagic discharge usually ceases during early metoestrus
- a mucoid vulval discharge may develop approximately 1 month into metoestrus, similar to that seen in pregnancy (see 12.5)
- pseudopregnancy (see 3.7) often occurs during late metoestrus

3.6 ANOESTRUS

- the period of ovarian inactivity between the end of metoestrus (or pregnancy) and the beginning of the next pro-oestrus
- this is normally approximately 4 months in duration but can be very variable, i.e. from 1 month to 2 years
- the length of anoestrus may be influenced by:
  (a) time of year: some bitches retain the primitive tendency for one period of pro-oestrus/oestrus a year, e.g. the Basenji breed; in other breeds more bitches may enter pro-oestrus/oestrus in spring than at other times
  (b) pheromones: bitches housed together often exhibit pro-oestrus/oestrus around the same time; it is thought that pheromones from one oestrous bitch may stimulate others
  (c) unknown factors: the signal responsible for causing follicular growth in most bitches is still unknown
- the advent of pro-oestrus may be predicted in some bitches by coat changes; where detection of the next pro-oestrus is important it may be sensible to collect a vaginal smear at a regular interval since the presence of red blood cells in the vaginal smear is predictive of the return to pro-oestrus

3.7 FALSE PREGNANCY (see also 19.8)

- synonyms include phantom pregnancy, pseudopregnancy and pseudocyesis
- false pregnancy is common and normal
- every non-pregnant bitch will have some clinical signs of false pregnancy

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- the clinical signs of false pregnancy are caused by elevated plasma prolactin concentrations
- classical signs include milk production and lactation, anorexia (but not weight loss), shivering, reluctance to leave the home, territorial aggression, nesting and mothering objects
- less common signs include minor changes in behaviour sometimes described as 'irritability'
- signs usually occurs 4–6 weeks after oestrus, but can occur at any time in some bitches
- represents a primitive mechanism for bitches to nurture other pups in the dog pack when pro-oestrus/oestrus occurred once a year, i.e. when the pack exhibited synchronized breeding
- the signs usually regress spontaneously after 10–30 days and in most bitches no medical treatment is required
- sometimes requires treatment if owners cannot cope with the clinical change in the bitch, if the signs are exaggerated (e.g. aggression) or if she develops mastitis
- there is only one episode of false pregnancy after each oestrus, but when hormone therapy is used in attempt to control the signs, repeated bouts may occur (see 27.1)
- although normal, false pregnancy is also discussed under endocrinological abnormalities (see 19.8)

Some myths about false pregnancy

Progesterone

- it is still said by some that progesterone is the cause of false pregnancy; this is not true because:
  (a) false pregnancy can be treated with progestogens (see 27.1)
  (b) bitches with false pregnancy do not have higher circulating concentrations of progesterone than others at the same stage of the cycle
  (c) bitches with false pregnancy do not produce progesterone for longer than others
  (d) bitches with false pregnancy do have raised prolactin blood concentrations
  (e) signs of false pregnancy can be suppressed by giving drugs that inhibit prolactin secretion, e.g. cabergoline (see 27.9)

Overt and covert false pregnancies

- it is often said that all bitches have a false pregnancy which in some is overt and in others is covert; this is not true and confusion has arisen due to the terminology used in other species
- all bitches have mammary swelling and the production of some milk

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- the clinical signs of pseudopregnancy encompass a large range with some bitches showing no signs and others exhibiting marked behavioural changes
- most cases can be considered physiological and normal and it is often the concern of the owner that governs which animals require treatment
- the situation in the cat and rat should not be confused with that in the dog: dogs are spontaneous ovulators (mating is not required)
- corpora lutea function for about 60–70 days in pregnant and non-pregnant bitches; in non-pregnant bitches this period of time is termed metoestrus or dioestrus (see 3.5)
- it is incorrect to refer to the luteal phase per se of the bitch as a false pregnancy
- clinical signs of false pregnancy do occur in some bitches during metoestrus; they are not directly related to progesterone concentrations in the blood

Pyometra

- it is sometimes said that bitches that have false pregnancies are more prone to pyometra; this is not true but:
  (a) false pregnancy and pyometra occur at the same stage of the cycle (metoestrus)
  (b) a bitch that has had progestogen treatments for false pregnancies may be more likely to develop pyometra as a result

Irregular oestrous cycles

- it is sometimes said that bitches with irregular oestrous cycles are more likely to develop false pregnancies; this is not true

Parity

- it is sometimes said that allowing a bitch to have a litter will stop subsequent false pregnancies; this is not true

Ovariohysterectomy

- it is sometimes thought that bitches which have signs of false pregnancy after ovariohysterectomy have ovarian tissue which has not been removed; this may be true but:
  (a) most false pregnancies that occur after ovariohysterectomy are the result of surgery just before or during a false pregnancy. Removal of the ovaries causes plasma progesterone concentration to decline and results in a surge release of prolactin
  (b) if a bitch does have a false pregnancy after ovariohysterectomy it is usually preceded by a period of oestrus if ovarian tissue is present

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REFERENCES

1 England, G.C.W., Allen, W.E. and Blythe, S.A. (1989) Variability of the time of calculated luteinising hormone release in 218 canine pregnancies. *Veterinary Record* **125**, 624–625.
2 England, G.C.W., Russo, M. and Freeman, S.L. (2009) Follicular dynamics, ovulation and conception rates in bitches. *Reproduction in Domestic Animals* **44**, 53–58.
3 England, G.C.W. (1994) Reproductive endocrinology in the dog and bitch. *Journal of Postgraduate Clinical Study In Practice* **16**, 275–279.

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4 Endocrinology of the Female

The previous chapter described the different phases of the oestrous cycle and of course these stages are determined by the hormonal changes that occur.

- hormone action is mediated by receptors on target tissues; these receptors change in distribution throughout the oestrous cycle [1]

4.1 GONADOTROPHIN-RELEASING HORMONE (GnRH, RARELY TERMED LHRH)

- released in a pulsatile manner in the part of the brain called the hypothalamus (Figure 4.1)
- reaches the anterior pituitary gland via a specialized (portal) blood circulation
- the hormone attaches to the cells which produce follicle-stimulating hormone (FSH) (see 4.2) and luteinizing hormone (LH) (see 4.3) to stimulate their production and release; it is thought that differences in pulse quality of GnRH select for FSH or LH release
- the effect of GnRH binding to the the receptors is transient because binding affinity is low; GnRH is rapidly released by the receptors and quickly metabolized
- synthetic analogues or agonists of GnRH (e.g. deslorelin) have been developed; these have increased potency due to their ability to remain attached to binding sites and to resist metabolism; after initial stimulation, long-acting agonists cause inactivity (down-regulation) of gonadotrophin-producing cells because pulsatile stimuli are prevented
- antagonists of GnRH have also been developed to compete for binding sites; these block endogenous activity but do not cause the initial stimulation seen with agonists (the agents are however expensive and have a number of adverse effects and have not been introduced into clinical practice)

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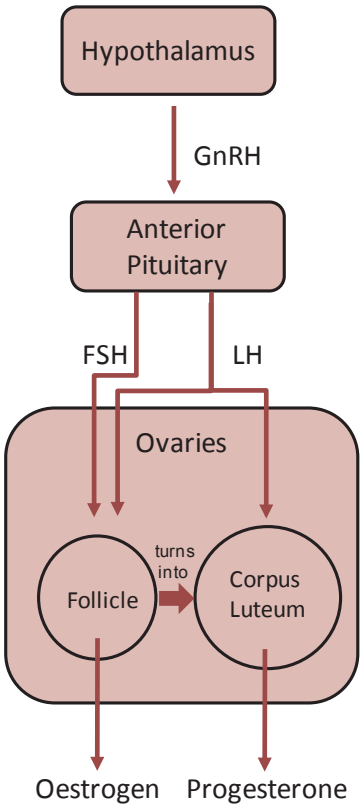


Figure 4.1 Schematic representation of the secretion of reproductive hormones in the bitch.

4.2 FOLLICLE-STIMULATING HORMONE (FSH)

- a gonadotrophin produced by and released from the anterior pituitary gland as a result of stimulation by GnRH
- it is responsible for the stimulation of the growth of follicles in the ovaries
- all factors which initiate its release are not known
- circulating concentrations in bitches are not well documented

4.3 LUTEINIZING HORMONE (LH)

- a gonadotrophin produced in and released from the pituitary gland as a result of GnRH stimulation
- it is released in short bursts or pulses
- increased pulse frequencies occur many weeks before the beginning of pro-oestrus [2]

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- LH stimulates the maturation, luteinization and ovulation of ovarian follicles
- circulating concentrations rise rapidly to reach a peak about 48 hours before the majority of ovulations (ovulations may occur over a 72-hour period)
- this peak occurs usually in late pro-oestrus or early oestrus (Figure 4.1)
- this hormone also has some luteotrophic action (maintains the function of corpora lutea)

4.4 OESTROGENS

- steroid hormones produced by growing follicles
- mainly 17alpha-oestradiol, 17beta-oestradiol and oestrone, some of which may be conjugated
- circulating oestrogen concentrations rise a little before the beginning of pro-oestrus
- a sharp rise in blood concentrations occurs later into pro-oestrus, resulting in a peak about 48 hours before the surge of LH (Figure 4.2)
- oestrogens cause a variety of clinical signs which occur in pro-oestrus:
  (a) vulval discharge (a result of uterine bleeding)
  (b) thickening of the vaginal mucosa and epithelium
  (c) change in cervical mucus consistency
  (d) vulval swelling
  (e) pheromone production
  (f) behavioural changes

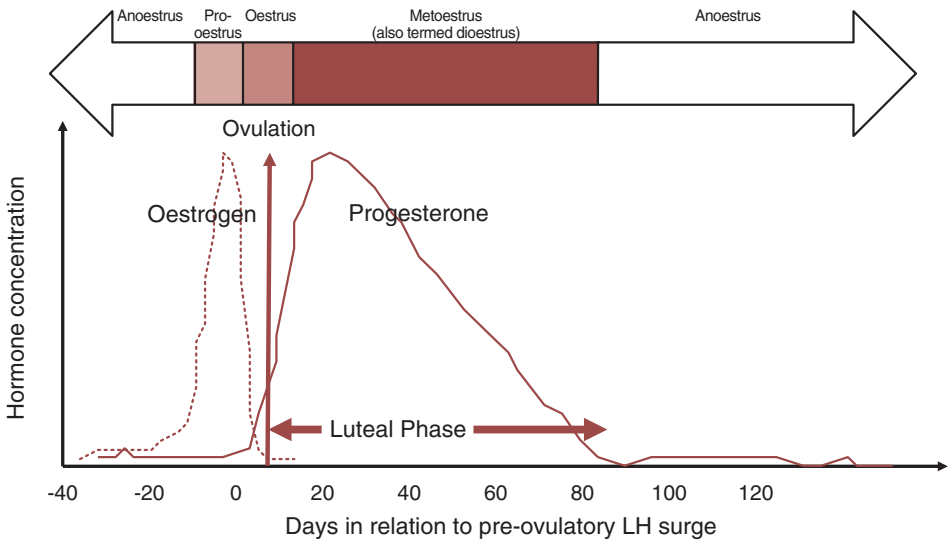


Figure 4.2 Schematic representation of the various stages of the oestrous cycle in relation to changes in hormone concentration.

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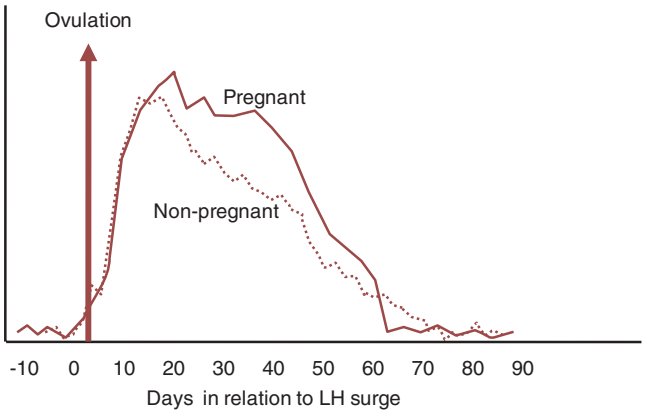


Figure 4.3 Schematic representation of the plasma progesterone profile during pregnancy and non-pregnancy.

4.5 PROGESTERONE [3]

- a steroid hormone produced by mature follicles (just before ovulation) and corpora lutea after ovulation and for the duration of their lifespan
- circulating concentrations start to rise around the time of the oestrogen peak (Figure 4.2); the hormone is produced by luteinizing granulosa cells in the unruptured follicles (this is unusual compared with many domestic mammals)
- it is possible to reliably and accurately measure bitch progesterone using ELISA techniques and these can detect the pre-ovulatory increase in progesterone [4]
- plasma values increase steadily and are significantly raised by the time of ovulation
- maximal concentrations are reached about 20 days after the end of oestrus, whether the bitch is pregnant or not
- a gradual decline in values then occurs until they become basal at about 60–70 days after ovulation
- the luteal phase is almost identical in pregnancy and non-pregnancy; in fact the non-pregnant luteal phase is slightly longer and progesterone concentration are slightly lower than in pregnancy (Figure 4.3); this is unique to the bitch among domestic species

4.6 PROLACTIN

- produced in and released by the anterior pituitary gland
- it acts on the mammary gland (primed by the actions of oestrogens and progesterone) to stimulate milk production
- blood concentrations usually rise as concentrations of progesterone fall

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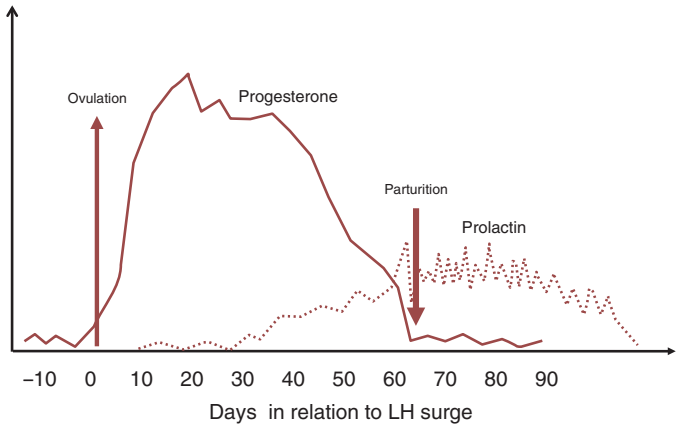


Figure 4.4 Schematic representation of the changes in hormone concentration during pregnancy.

- prolactin causes behavioural changes typically seen in late pregnancy and lactation
- in nursing bitches, blood concentrations of prolactin are very high for some time after parturition (Figure 4.4)
- prolactin causes signs of pseudopregnancy (see 3.7 and 19.8)
- prolactin is also luteotrophic (maintains the function of corpora lutea); the luteotrophic action of prolactin is often overlooked or poorly understood but this role of prolactin is very important when considering pharmacological methods that are available to manipulate the cycle of the bitch (see 27.9)

4.7 ANDROGENS

- the functions of these hormones in the bitch are largely unknown
- testosterone concentrations in the blood peak at the same time as LH during oestrus
- androstenedione concentrations are high during metoestrus and pregnancy, and closely follow those of progesterone

4.8 PROSTAGLANDINS

- in many species prostaglandin is produced by the uterus in non-pregnant females to terminate the luteal phase and cause a return to oestrus
- in the bitch the length of the luteal phase of non-pregnancy is similar to the length of the luteal phase of pregnancy and prostaglandin is not responsible for terminating the luteal phase and causing a return to oestrus

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- in the non-pregnant bitch corpora lutea appear to become non-functional gradually, due either to lack of trophic support (possibly LH and/or prolactin) or because they have an inherent lifespan
- in the pregnant bitch, however, prostaglandin causes final lysis of the corpora lutea and the initiation of parturition. At this time the corpora lutea are producing very little progesterone compared with earlier in pregnancy
- the mechanisms of action of prostaglandin in pregnant bitches appears to be similar to that seen in other species
- exogenous administration of prostaglandin can be used to terminate the luteal phase of pregnancy or non-pregnancy, although there are some interesting requirements for prostaglandin administration to produce this effect

REFERENCES

1 Dhaliwal, G.K., England, G.C.W. and Noakes, D.E. (1997) Immunocytochemical localisation of oestrogen and progesterone receptors in the uterus of the normal bitch during oestrus and metoestrus. *Journal of Reproduction and Fertility* **51**(Supplement), 167–176.

2 Jeffcoate, I.A. and England, G.C.W. (1997) Urinary LH, plasma LH and progesterone and their clinical correlates in the periovulatory period of domestic bitches. *Journal of Reproduction and Fertility* **51**(Supplement), 267–275.

3 England, G.C.W. (1994) Reproductive endocrinology in the dog and bitch. *Journal of Postgraduate Clinical Study In Practice* **16**, 275–279.

4 England, G.C.W., Allen, W.E. and Porter, D.J. (1989) A comparison of radioimmunoassay with quantitative and qualitative enzyme-linked immunoassay for plasma progestogen determination in bitches. *Veterinary Record* **125**, 107–108.

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5 Clinical Examination of the Female

Clinical examination of the reproductive tract is the mainstay of investigation of normality and abnormal function. A logical ordered approach to the examination is essential to ensure that no important features are overlooked.

5.1 BASIC EXAMINATION OF THE REPRODUCTIVE TRACT

This consists of:

- visual examination of the perineum and vulva
- palpation of the mammary glands
- collection of vaginal cells for the study of vaginal exfoliative cytology
- digital examination of the vestibule and caudal vagina
- endoscopic examination of the vestibule, vagina and cervix
- real-time ultrasonographic imaging
- measurement of plasma hormone concentrations

5.2 ADDITIONAL TECHNIQUES THAT MAY BE CONSIDERED

These include:

- bacteriological examination of the reproductive tract
- endocrinological stimulation tests
- cytological examination of suspected pathological tissue
- laparoscopy or laparotomy techniques
- karyotyping

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5.3 VISUAL EXAMINATION OF THE PERINEUM

- allows confirmation of anatomic normality or abnormality
- may allow the stage of the oestrous cycle to be predicted (swelling occurs during pro-oestrus and oestrus)
- may allow the detection of intersex abnormalities (see Chapter 18)

5.4 PALPATION OF THE MAMMARY GLANDS

- mammary glands and nipples are small prior to puberty
- mammary glands are usually small during anoestrus and pro-oestrus
- mammary gland size and nipples may be large in multiparous bitches
- approximately 35 days after ovulation there is increased size of the mammary glands:
  (a) at this time there is often milk production
  (b) pseudopregnancy is common and normal
- it is often feasible to estimate the stage of the oestrous cycle by palpation of the mammary glands

5.5 VAGINAL EXAMINATION

Vaginal cytology

To understand the changes in vaginal cytology it is helpful to consider the histological changes in the vaginal epithelium:

- it is critically important to implement appropriate quality control measures in any diagnostic laboratory; work in the author's laboratory was the first to consider quality assurance measures for canine reproductive laboratories and to document levels of appropriate control that can be achieved [1]

Changes throughout the oestrous cycle

Prepuberty and anoestrus

- epithelium consists of two layers, the outer of which appears pseudostratified
- there is no leucocytic infiltration

Pro-oestrus

- the epithelium gradually becomes stratified squamous of approximately 16 cells in thickness at the end of pro-oestrus
- hyperplasia, hypertrophy and mitoses are seen in the basal layers

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- the superficial squamous cells become large and irregular in outline and are usually keratinized
- there is no leucocytic infiltration

Oestrus

- the stratified squamous epithelium persists throughout the fertile period
- there is no leucocytic infiltration

Metoestrus (dioestrus)

- the squamous epithelium starts to slough abruptly approximately 7 days after ovulation when progesterone concentrations reach high values
- the epithelium returns to a smaller number of layers often between three and six cells
- the epithelium and lamina propria are infiltrated with polymorphonuclear neutrophils
- by mid metoestrus the epithelium returns to two cells in thickness and cellular infiltration has subsided

Examination of vaginal cytology

- cells may be collected by a variety of different methods before staining and microscopic examination; the methods are described below
- the appearance of cells collected can be used to determine the various stages of the oestrous cycle (Figure 5.1) as well as the optimal time for breeding

Anoestrus

- epithelial cells: these are mainly round cells with active nuclei and a small cytoplasm. The smaller cells (parabasal cells) originate close to the basement membrane of the mucosa; the larger cells are produced by cell division and are known as (small) intermediate cells
- polymorphonuclear neutrophils: usually present in small to moderate numbers
- erythrocytes: absent
- bacteria: usually absent

Pro-oestrus

- epithelial cells: immediately before, and also during, early pro-oestrus the number of large intermediate cells (with an increased cytoplasmic:nuclear ratio compared with the small intermediate cells) in the smear increases
- towards the end of pro-oestrus there is an increase in the proportion of cells which are large and irregular in outline and which become keratinized, i.e.

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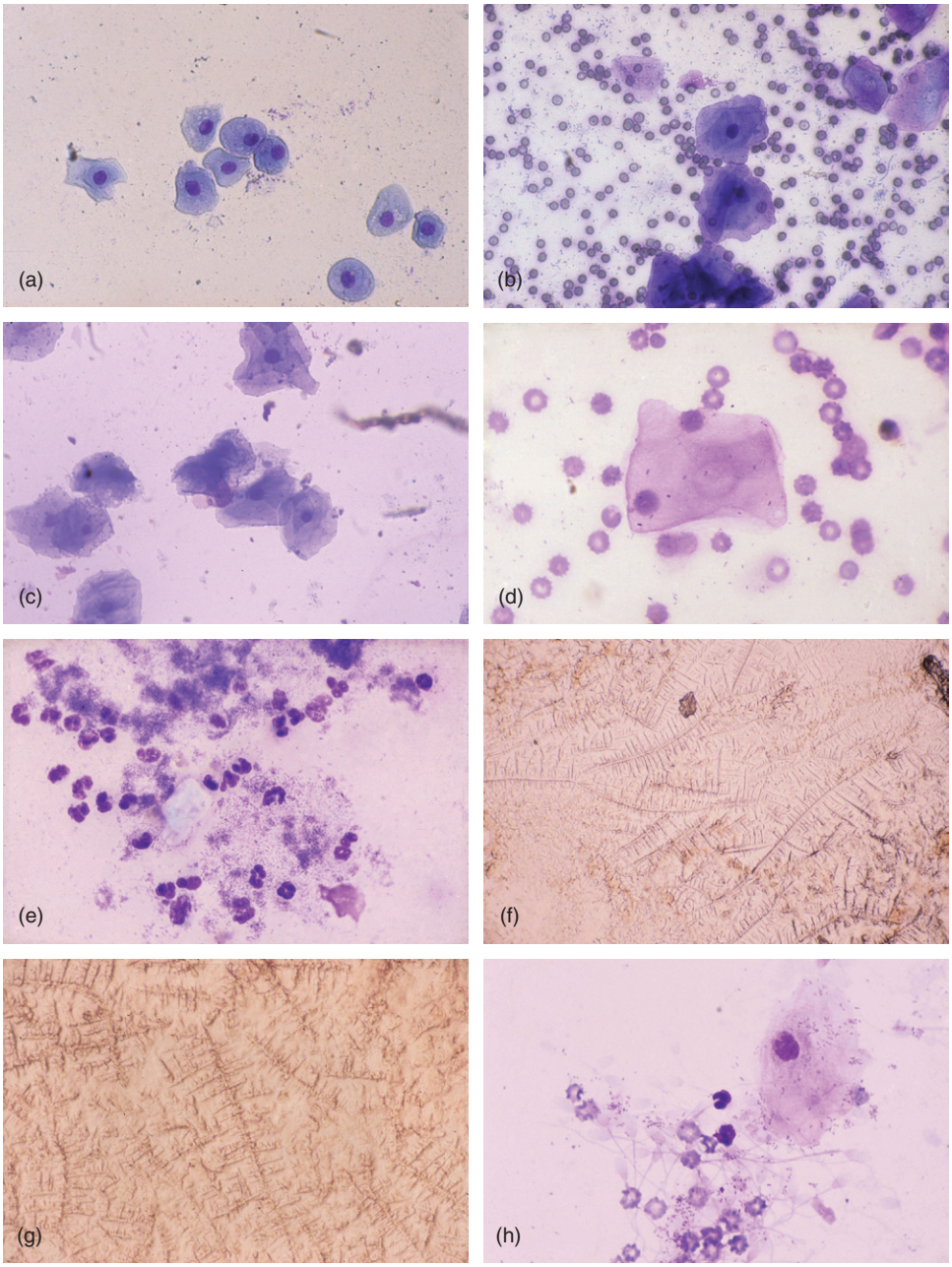


Figure 5.1 Vaginal smears collected by aspiration and stained with 'Diff-Quik'. (a) Pro-oestrus: characterized by small intermediate epithelial cells. (b) Early oestrus: characterized by large intermediate epithelial cells (large numbers of erythrocytes are also present). (c) Oestrus: characterized by large intermediate epithelial cell which are now very large and irregular in outline. (d) Oestrus: at the optimal time of mating the majority of epithelial cells are anuclear. (e) Metoestrus/dioestrus: characterized by cellular debris and large number of polymorphonuclear neutrophils. (f) Unstained smear from a bitch in pro-oestrus showing forming crystallization pattern. (g) Unstained smear from a bitch in oestrus showing significant crystallization pattern. (h) Vaginal smear post mating showing spermatozoa, two polymorphonuclear neutrophils, erythrocytes and a single large intermediate epithelial cell.

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appear straight-sided with smaller nucleus and where keratin can be detected with specific staining
- polymorphonuclear neutrophils: present in small numbers initially but gradually decrease; usually PMNs are absent at the end of pro-oestrus
- erythrocytes: usually present in large numbers throughout pro-oestrus, however the number is of no clinical significance; sometimes they are lysed during preparation of the vaginal smear
- bacteria: often present and increase in number throughout pro-oestrus

Oestrus

- critical information relating to the clinical application of vaginal cytology has been published from the author's laboratory that has led to wide understanding and application of the technique [2,3]
- epithelial cells: early in oestrus maximal cornification (keratinization) occurs, i.e. between 60 and 90% of cells are straight sided and have small (pyknotic) nuclei or no nucleus (they are then called squames)

NB: A 'false' or early peak of cornification sometimes occurs before the real peak; this can be recognized by the vague (pale staining) nature of the squames – the cause of this is unknown but it is not reported by those who use trichrome stains.

- polymorphonuclear neutrophils: usually absent throughout oestrus
- erythrocytes: may be absent, few or many; numbers are of no clinical significance
- bacteria: present usually in large numbers until the end of oestrus

Metoestrus (dioestrus)

- epithelial cells: at the end of oestrus there is a sudden change to all the epithelial cells becoming intermediate or parabasal, and present in large numbers; as metoestrus proceeds cell numbers decrease and cells are similar to those in anoestrus
- polymorphonuclear neutrophils: at the end of oestrus there is a massive influx of these cells; as metoestrus proceeds, numbers decrease but some are always present
- erythrocytes: usually absent but may be seen in early metoestrus and are of no clinical significance
- bacteria: disappear dramatically at end of oestrus, coincident with influx of neutrophils and the appearance of mucus and debris
- there is commonly a significant amount of mucus present in the vaginal smear of bitches in metoestrus

NB: Vaginal smears are similar in metoestrus and pregnancy.

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Preparation of vaginal smears

- collection of vaginal material can be achieved in a number of ways

Bacteriological swab

- insert a bacteriological swab into the vestibule and caudal vagina to absorb exudate; gently roll the swab onto a microscope slide
- this method is not ideal because:
  (a) vestibular cells are also collected
  (b) in metoestrus and anoestrus mucus is collected; this dries (fixes) slowly on the slide and causes distortion of cells
  (c) the physical rolling of the swab on the slide causes cell disruption
  (d) elements from the swab are included in the smear
- the method can be improved by using a speculum or cylindrical guide to prevent contamination with vestibular cells

Spatula

- scrape material from the vaginal wall using a metal spatula
- this method is not ideal because:
  (a) very little material is collected
  (b) the material is difficult to spread over the microscope slide
  (c) the degree of pressure in scraping may result in deeper or more superficial layers of cells being collected

Aspiration

- aspirate material from the caudal vagina using a catheter or pipette; place a drop of exudate on a microscope slide and spread as a blood smear
- during metoestrus and anoestrus a small amount of saline can be introduced into the vagina and re-aspirated, to collect a suspension of cells; if the smear is made and dried immediately, cell distortion does not occur
- this method produces a clear undistorted smear, and the collected material can also be used for examination of mucus crystallization in oestrus (see 11.3)
- in the author's view this method is ideal

Staining vaginal smears

- can be achieved in a number of ways

Shorr's method

- using three different stains to produce a trichrome technique
- advantages:

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  (a) differentiates between keratinized (orange) and non-keratinized (blue) epithelial cells
  (b) from this an index of keratinization (eosinophilic index) can be calculated
- disadvantages:
  (a) there is no absolute value of the eosinophilic index which indicates ovulation time
  (b) the staining method is complicated and very time consuming if individual stains are used, and components require regular filtering or renewal

NB: Trichrome stains like Shorr's are excellent for human vaginal cytology where many slides are processed each day; bitch vaginal smears are usually taken too infrequently to justify the use of a complicated staining technique.

Methylene blue

- using a single stain which is poured onto an air-dried slide for 5–20 minutes before being washed with water before immediate examination
- advantages:
  (a) very simple
  (b) rapid
- disadvantages:
  (a) must examine the wet slide immediately, as drying causes distortion of the cells
  (b) examining wet slides under high power may cause condensation on the objective lens
  (c) no permanent record of the slides can be kept

Leishman's stain

- using a single stain to flood the air dried for 2–3 minutes before adding 2 ml of phosphate buffer gently so that surface tension prevents dripping; a metallic sheen will be seen on the surface of the diluted stain which is washed off after 20 minutes before drying and examination
- advantages:
  (a) relatively simple
  (b) medium speed of preparation
- disadvantage:
  (a) the long staining step sometimes results in the sample being left for too long and therefore drying out

Rowmanowski stains (e.g. Diff-Quik)

- using a fixative and two stains the air-dried slide is dipped sequentially six times into each of the three solutions, washed immediately with water and allowed to dry before examination

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- advantages:
  (a) rapid
  (b) gives consistent results
- disadvantages:
  (a) expensive
  (b) one solution (fixer) evaporates rapidly (even if not used)
  (c) solutions must be changed frequently

Interpretation of vaginal smears

There is a variety of uses of vaginal smears but commonly this is to establish the stage of the oestrous cycle or for the demonstration and identification of abnormal cells.

Determination of optimal mating time (see also Chapter 11)

- vaginal cytology is an important indirect method of monitoring hormonal events (particularly elevation of plasma oestrogen – see 3.4); the scope for anticipating future changes is limited
- vaginal cytology can only be useful for indicating the fertile period and not the fertilization period; i.e. the broad period of time when mating or insemination is likely to result in pregnancy (see 11.2)
- the first smear should be taken approximately 5 days after the first observed signs of pro-oestrus, as some bitches may be close to ovulation at this time
- depending on the cellular content, repeat the smears every 2–3 days, unless it is clear that the bitch is very early in pro-oestrus
- to calculate the percentage of anuclear cells:

$$\text{anuclear cell index} = \frac{\text{number of anuclear cells}}{\text{total number of epithelial cells examined}} \times 100$$

- mating should be recommended when usually more than 80% of cells are anuclear and there is an absence of polymorphonuclear neutrophils (Figure 11.4) [2]
- the sudden decrease in the anuclear cell index, and a return of a large number of polymorphonuclear neutrophils denotes the end of the fertilization period

Diagnosis of vaginitis (see 16.3 and Figure 5.1)

- excess vulval discharge at a time when no discharge is normally present suggests vaginal or uterine disease
- male dogs are often interested in bitches with vulval discharge, even when they are not in oestrus
- it is difficult to define vaginitis in the bitch because there are polymorphonuclear neutrophils present at all stages except during oestrus

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- very large numbers of polymorphonuclear neutrophils in vaginal smears suggest uterine disease (pyometra: endometrial cells may also be seen)
- many vulval discharges are normal, i.e. during metoestrus and pregnancy, and may simply indicate increased mucus production
- many bitches considered to have excessive vulval discharge have very few polymorphonuclear neutrophils in vaginal smears and are probably normal
- bacteriological findings are discussed later
- vaginal smears are invaluable for distinguishing bitches which are in pro-oestrus/oestrus from bitches which are otherwise attractive to male dogs

Pregnancy diagnosis

- vaginal smears cannot be used as a method of pregnancy diagnosis
- smears will not distinguish between metoestrus, pregnancy and anoestrus

Crystallization (ferning) patterns in vaginal (cervical) mucus (Figure 5.1f,g)

- there is little use of this technique in veterinary practice, but it can provide additional information to demonstrate the stage of the oestrous cycle, especially oestrus and the fertile period
- work from our laboratory has demonstrated that this technique is simple and effective at documenting the broad time of the fertile period [3]

Collection of samples

- vaginal secretion is best collected by pipette or catheter as described earlier
- a drop of the exudate is placed onto a microscope slide and held vertically to allow the drop to 'run'
- do not examine until the slide is completely dry
- examine under ×40 objective with the condenser lowered to enable greatest contrast of material

Interpretation of mucus crystallization

- the smear is examined for both the type of ferning pattern and the area of the smear covered by this pattern
- the ferning pattern is scored arbitrarily using five classes from minor ferning (with short stems and irregular stellate patterns) to bold ferning (with long stems and clear veination and subveination)
- the surface area is scored using five classes from less than 1% cover, to greater than 20% cover
- the two scores may be added to calculate the ferning index (values 0–10)

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- ferning is most intense and covers a greater area of the smear after the second peak of cornification of epithelial cells; it may therefore help differentiate the first and second peak [2]
- individual bitches do not always reach a peak ferning index score of 10
- vaginal washings will give erroneous results due to the addition of water; with this method, crystallization can be seen in metoestrous samples

5.6 DIGITAL EXAMINATION OF THE REPRODUCTIVE TRACT

- clean the vulva of gross contamination and then prepare the area using an antibacterial preparation such as a surgical hand cleaner
- gently insert a lubricated gloved finger
- the vestibule is directed upwards to the vagina
- the external urethral orifice can be palpated on the ventral surface at the vestibulo-vaginal junction
- the vagina is dry in anoestrus and metoestrus (dioestrus), but moist during pro-oestrus and oestrus
- it is possible to detect hymenal remnants or abnormalities of the vestibulo-vaginal junction (strictures, see 16.3)
- it is possible to detect hyperplasia of the vaginal floor during oestrus (see 16.3)

5.7 ENDOSCOPIC EXAMINATION OF THE FEMALE REPRODUCTIVE TRACT

- bitches in oestrus may allow endoscopic examination with or without sedation; at other times sedation or general anaesthesia may be necessary
- auroscopes, even with long nozzles, are too short for thorough examination
- most bitch vaginal speculae also only reach to the vestibulo-vaginal junction
- rigid cold-light endoscopes and those with flexible fibreoptics give best results but:
  - (a) it may be difficult to focus on the vaginal wall unless the canal is distended with air or water
  - (b) the caudal limit of the dorsal median fold may be mistaken for the cervix
  - (c) the cranial half of the vagina is very narrow, especially in the non-oestrous bitch, and may be difficult to visualize (see Figure 2.2)
  - (d) on many occasions unless the bitch is in oestrus the vaginal os of the cervix will not be seen
  - (e) examination to elucidate the cause of abnormal discharge is usually unrewarding because the discharge may flood the field of view; in these cases lavage with volumes of saline may be helpful
- vaginal endoscopy may be useful for the assessment of the optimal mating time (see Chapter 11) and certain cases of reproductive tract disease

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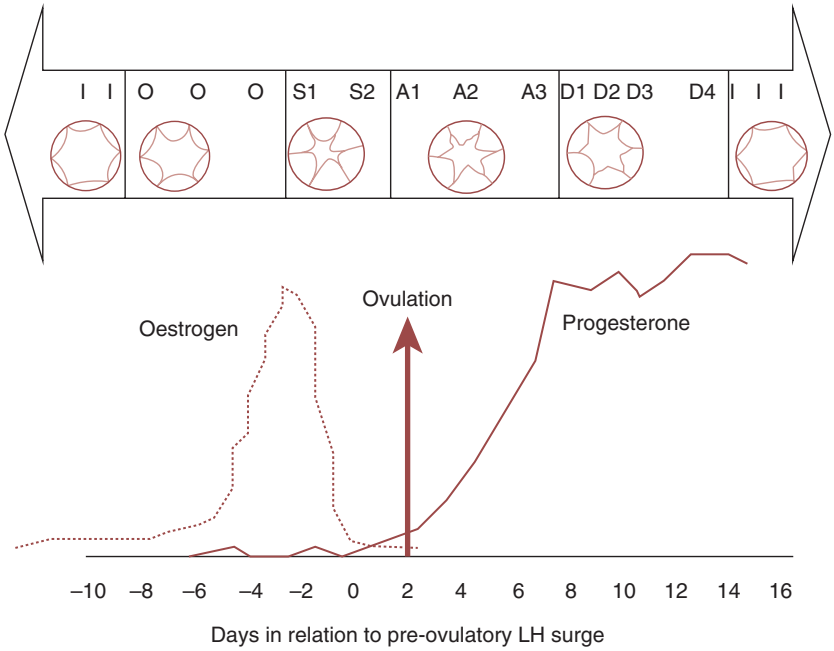


Figure 5.2 Schematic representation of the changes in endoscopic appearance of the vagina in relation to the hormonal profile at different stages of pro-oestrus, oestrus and metoestrus (dioestrus).

Interpretation (Figure 5.2)

General changes

- anoestrus: vaginal folds are flat and may disappear when the vagina is insufflated; they appear red due to the underlying capillaries and overall the mucosa is dry
- pro-oestrus: vaginal folds become larger, rounder, moister and paler; sanguinous discharge is seen between the folds
- early oestrus: the folds become wrinkled and subdivided into smaller rounder folds
- late oestrus: the secondary folds become angulated and the vagina appears dry
- metoestrus: the folds become rounded and less distinct and the mucosa appears patchy due to loss of the cornified layer

Quantification of changes to establish the optimal time to breed (see also Chapter 11)

- bitches in anoestrus have a red and dry mucosa with low and flattened mucosal folds; this is described as the inactive phase (I)

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- increased oestrogen causes thickening and oedema of the mucosa which appears turgidly swollen, rounded and grey/white in colour; this is described as the oedematous phase (O)
- a decrease in oestrogen and rise in progesterone causes reduced turgidity with progressive furrowing, wrinkling and indentations; this is described as the early and late shrunken phase (S1, S2)
- progressive increased progesterone causes progressive shrinkage and angulation such that the peaks are sharp-tipped and irregular in appearance; this is described as the early, middle and late angulation phase (A1, A2, A3)
- after the end of the fertilization period when progesterone concentrations are high there is a progressive decline in the size of the mucosal fold profile; this is designated as the early, middle and late decline phase (D1, D2, D3, D4)
- the decline phase is followed by a return to a phase characterized by a thin, red and dry mucosa with low and flattened mucosal folds; the inactive phase (I)

5.8 ULTRASONOGRAPHIC EXAMINATION OF THE FEMALE REPRODUCTIVE TRACT

Principles of diagnostic ultrasound

- diagnostic ultrasound utilizes sound frequencies between 2 and 10 MHz
- ultrasound is produced by application of an alternating voltage to piezoelectric crystals which change in size and produce a pressure or ultrasound wave. Returning echoes deform the same crystals which generate a surface voltage
- most diagnostic ultrasound machines use the principle of brightness modulation (B-mode) where the returning echoes are displayed as dots, the brightness of which is proportional to their amplitude
- real-time B-mode ultrasound is a dynamic imaging system where information is continually updated and displayed on a monitor
- ultrasound is attenuated within tissues and attenuation is related to wavelength of the sound, the density of the tissue, the heterogenicity of the tissue and the number and type of echo interfaces
- bright (specular) echoes are produced when a large proportion of the beam is reflected back to the transducer; these echoes are displayed as white areas on the ultrasound machine screen
- no echoes are produced when the sound is transmitted and not reflected; these areas are displayed as black on the ultrasound machine screen
- non-specular echoes are produced when the beam encounters a structure similar to one wavelength in size, and these echoes appear as varying shades of grey on the ultrasound machine screen

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The ultrasound transducer

- piezoelectric crystals are arranged together to form an ultrasound transducer, contained within the ultrasound head
- transducers produce sound of a characteristic frequency:
  (a) high frequencies allow good resolution although there is greater attenuation of the sound beam in tissues
  (b) low frequencies allow a greater depth of penetration (less attenuation) but with reduced resolution

Equipment for examination of the bitch

- sector or micro-convex transducers are most suited to transabdominal imaging
- for the examination of the ovaries and of early pregnancy a 7.5 MHz transducer is most suitable
- for the examination of later pregnancy a 5.0 MHz transducer is necessary
- a 5.0 MHz transducer offers a compromise which gives a reasonable depth of penetration combined with adequate tissue resolution
- the ultrasound machine should have a keyboard to allow identification of the animal and possess electronic callipers to allow measurement of images
- there should be facilities to record the images and this can be achieved using:
  (a) a thermal printer
  (b) digital frame recording

Ultrasound terminology

- tissues that markedly reflect sound (such as gas, bone and metal) appear white on the ultrasound screen and are called echogenic
- tissues that transmit sound (such as fluid) appear black on the ultrasound screen and are called anechogenic (or anechoic)
- tissues that allow some transmission and some reflection (such as most soft tissues) appear as varying shades of grey and are called hypoechogenic or hyperechogenic depending upon their exact appearance
- strictly, a hyperechogenic tissue produces a hyperechoic region within the image, although these terms are often used synonymously

Examination of the bitch

- clipping the hair of the ventral abdomen is required for examination of the uterine body and horns
- clipping of the hair over the flank is often necessary to image the ovaries
- in many patients imaging is most conveniently performed with the bitch in the standing position
- the skin should be cleaned of debris and normally alcohol is applied which helps to remove debris and increase the contact of the ultrasound gel

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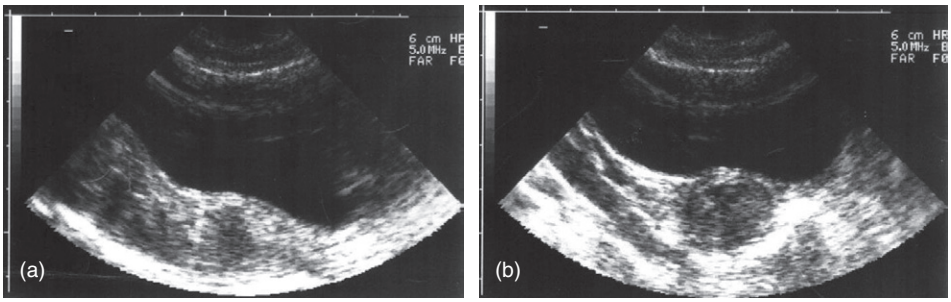


Figure 5.3 Uterine body of a bitch during oestrus: (a) longitudinal ultrasound image, the uterus lies dorsal to the anechoic bladder (ventral in this image since the bitch was in the standing position with the transducer adjacent to the ventral abdominal wall), and has a rectangular appearance; (b) transverse ultrasound image, the uterus has a circular appearance and the hypo- and hyperechoic layers can be easily identified (5.0 MHz transducer, scale in centimetres).

The normal non-pregnant uterus

- lies dorsal to the bladder, but its position may be variable depending upon:
  (a) the extent of bladder filling
  (b) the size of the uterus
  (c) the stage of the reproductive cycle
- the uterine body of the pre-pubertal and non-pregnant bitch during anoestrus may be less than 1 cm in transverse diameter
- the uterus may be identified dorsal or dorso-lateral to the bladder, and can be shown to be tubular by imaging in two perpendicular planes (Figure 5.3)
- the uterus may be more readily imaged in older multiparous bitches
- in older bitches the bifurcation and distal uterine horns can be detected in approximately 40% of cases
- the uterine body and horns are composed of two distinct layers (Figure 5.3):
  (a) a central homogenous relatively hypoechoic region (endometrium)
  (b) a peripheral hyperechoic layer (serosa)
- in the author's laboratory we have described the detail of the ultrasonographic appearance of the uterus throughout the oestrous cycle [4]
- during oestrus the uterus becomes increasingly hypoechoic and develops central radiating hyperechoic lines
- the changes in uterine echotexture are associated with an increase in uterine diameter
- the changes in uterine echogenicity and size are not sufficiently specific to allow the anticipation of ovulation time
- the echotexture returns to a homogenous nature during metoestrus (dioestrus)
- uterine transverse section diameter continues to increase during early metoestrus
- uterine diameter increases in both pregnant and non-pregnant bitches

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- pregnancy diagnosis requires the detection of fluid-filled (anechoic) conceptuses (see 13.9)

The normal ovary [4]

- some of the first reports on the ultrasonographic appearance of follicles and corpora lutea were published from the author's laboratory [5,6]
- ovaries are difficult to examine due to their small size and superficial location
- during anoestrus the ovaries are located adjacent to the caudal pole of the kidney close to the lateral abdominal wall
- during oestrus the ovaries are often located more caudally and ventrally
- during anoestrus the ovaries of the bitch are less than $1.5 \times 1.0 \times 0.8$ cm in size, and are relatively homogenous
- at the onset of pro-oestrus small fluid-filled anechoic follicles may be detected within the ovarian stroma
- when first imaged the internal diameter of follicles is between 1 and 2 mm
- follicles enlarge during pro-oestrus and become less spherical in outline
- the maximum follicular diameter is usually attained 1 day after the surge of plasma LH, i.e. the day before ovulation
- anechoic follicles are replaced by hypoechoic structures (presumably early corpora lutea) between 1 and 3 days after the surge of plasma LH; however, these structures are present only transiently, before being replaced by anechoic structures with 2 mm thick hypoechoic walls
- luteal structures are most commonly cavitated during early metoestrus (dioestrus)
- the time of ovulation is difficult to demonstrate without repeated examination of the bitch but in work from the author's clinic detailed descriptions of follicular dynamics and appearance of the corpora lutea was published [7]
- the detection of ovulation with ultrasound has limited value, especially when compared with more simple techniques such as vaginal cytology and measurement of plasma progesterone concentration (Figure 5.4); however recent

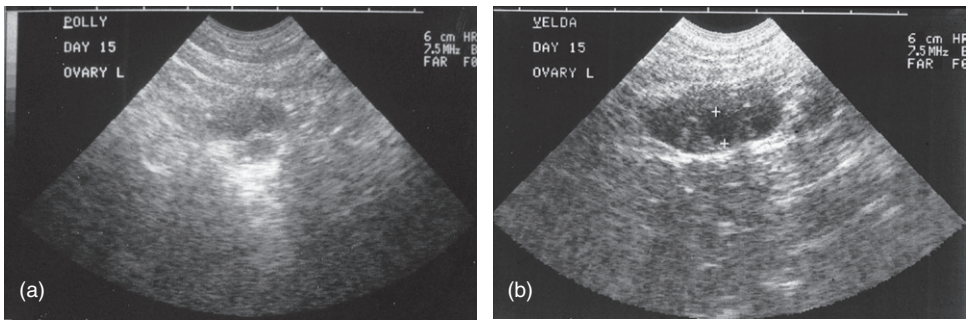


Figure 5.4 Ultrasound image of a bitch ovary: (a) during oestrus, three small circular anechoic follicles are present; (b) during early metoestrus (dioestrus), three thick-walled anechoic corpora lutea are present (5.0 MHz transducer, scale in centimetres).

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understanding of follicle dynamics and documentation of abnormal ovarian function with ultrasound [7] will undoubtedly lead to improved clinical practice in the future

5.9 MEASUREMENT OF PLASMA HORMONES (see Figure 4.1)

- measurement of the plasma concentrations of the steroid hormones may be useful for estimating the stage of the oestrous cycle
- conventionally hormone concentrations are measured by radioimmunoassay [8]; however
  (a) these are often costly
  (b) samples are often only assayed in batches
  (c) there is often a delay in receiving the results
- hormone concentrations may be accurately measured using enzyme-linked immunosorbent assay (ELISA) techniques:
  (a) these can be performed in the practice laboratory
  (b) results can usually be obtained within 1 hour of sample collection
- in the UK both quantative and qualitative ELISA methods are available for the assessment of progesterone concentration:
  (a) quantitative techniques require the use of a plate reader and plotting of a standard curve for known progesterone standards
  (b) qualitative techniques allow the estimation of progesterone concentration by comparison of a colour change with known progesterone standards
- we have documented the technical and financial perspectives relating to these methods [9]; this is important and this type of work will affect utilization of techniques in clinical practice

Plasma (serum) progesterone concentration (Figure 5.5)

- progesterone is produced solely by the pre-ovulatory follicle and the corpus luteum in the bitch
- progesterone concentrations increase immediately before ovulation
- progesterone concentrations peak approximately 10–15 days after ovulation
- serial monitoring of plasma progesterone therefore allows:
  (a) anticipation of ovulation
  (b) confirmation of ovulation
  (c) detection of the fertilization period (see Chapter 11)
  (d) the likely time of onset of parturition
- values decrease slowly throughout the luteal phase

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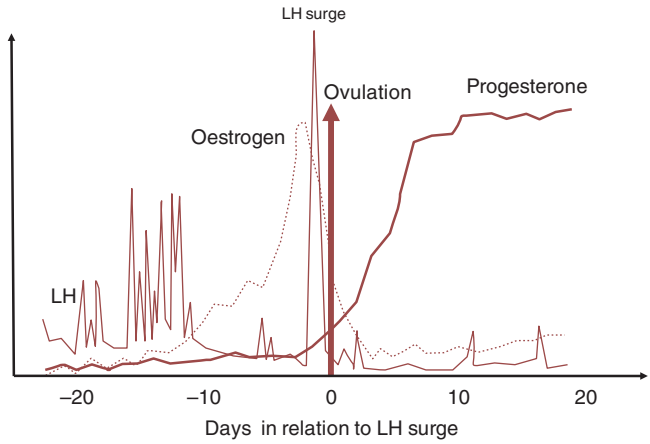


Figure 5.5 Changes in plasma progesterone at the time of the LH surge and ovulation in the bitch.

- progesterone concentrations are similar in pregnancy and non-pregnancy (non-pregnant values are lower, but because of individual variation values cannot be used to diagnose pregnancy or non-pregnancy)
- the non-pregnant luteal phase lasts approximately 68 days from ovulation
- the pregnant luteal phase lasts approximately 63 days from ovulation
- there is a sudden decrease in progesterone 24–36 hours before parturition

NB: It is feasible to run ELISA on whole blood and vaginal fluid which removes the need for centrifugation of the sample and therefore increases its practical application in some clinical situations [10,11].

Interpretation

Stage of cycle	Progesterone concentration
Anoestrus	Basal – less than 1.0 ng/ml (less than 3.0 nmol/l)
Pro-oestrus	Basal – less than 1.0 ng/ml (less than 3.0 nmol/l)
Oestrus	Basal – less than 1.0 ng/ml (less than 3.0 nmol/l)
Day of LH surge	Intermediate – 2.0 ng/ml (6.5 nmol/l)
Day of ovulation	Intermediate – 5.0 ng/ml (15 nmol/l)
End of fertilization period	High – 8.0–10.0 ng/ml (25.0–32.0 nmol/l)
Non-pregnant luteal phase	High early, intermediate later
Pregnant luteal phase	High early, intermediate later
24 hours before parturition	Low

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Plasma (serum) oestrogen concentration

- oestrogen concentrations during the majority of anoestrus are low
- concentrations increase in late anoestrus and throughout pro-oestrus
- peak values are reached during late pro-oestrus or early oestrus, approximately 2 days before the LH surge
- concentrations are low at the time of ovulation
- concentrations during oestrus may therefore be high or low depending upon when a sample is taken in relation to ovulation
- oestrogen concentrations increase during the late non-pregnant and late pregnant luteal phase
- measurement of resting plasma oestrogen concentration has little clinical indication in the female

Plasma (serum) luteinizing hormone (LH) concentration

- concentrations of LH increase from 1 month before ovulation
- there is a large pre-ovulatory LH surge (values increase by 20 to 40 times) which appears to be the trigger for ovulation [12]
- the pre-ovulatory surge occurs approximately 1–2 days after peak values of oestrogen are reached
- the pre-ovulatory surge lasts 24–72 hours
- serial sampling and the detection of the LH surge therefore allows:
  (a) anticipation of ovulation
  (b) detection of the fertilization period (see Chapter 11)
- LH is, however, only reliably measured by radioimmunoassay at present
- LH is luteotrophic and is elevated similarly in the pregnant and non-pregnant luteal phase
- LH concentrations are very high in neutered animals and can be used to demonstrate absence of gonads if such assays are readily available

5.10 BACTERIAL EXAMINATION OF THE FEMALE REPRODUCTIVE TRACT

Normal bacterial colonization

Vestibule and caudal vagina

- these have a normal bacterial flora
- this changes daily
- many species of bacteria are present, including haemolytic streptococci, other streptococci, staphylococci, Escherichia coli and many others
- ureoplasmata and mycoplasmata are also normal
- there is usually a mixed flora, with two to five species present

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- the prevalence of anaerobic bacteria is not well documented but these are certainly frequently isolated when attempts are made
- Pseudomonas spp. are not commonly isolated from normal bitches
- there is no correlation between the numbers of organisms cultured and those seen in vaginal smears; this suggests that some stained bacteria may be dead or are anaerobes

Cranial vagina

- there is little information on the flora of the cranial vagina and cervix
- vagino-cervical defence mechanisms, i.e. mucus production with discharge to the vulva and neutrophil mobilization, may normally discourage bacterial colonization of the cranial vagina

Uterus

- the uterus is usually thought to be bacteriologically sterile except in oestrus or after parturition when the cervix opens
- however:
  (a) one study has isolated organisms from apparently normal uteri at other stages of the cycle
  (b) it is inevitable that vaginal flora will be introduced into the uterus with the ejaculate during coitus and
  (c) in post-partum metritis and pyometra bacteria of vaginal origin appear to be capable of contributing to the disease process
- it is possible to collect samples from the uterus using endoscopic techniques; however, these are liable to contamination from the cranial vagina and results should be interpreted cautiously

Interpretation of vaginal bacteriology

- most swab samples are taken from the vestibule
- they should therefore be expected to contain bacteria
- there is no evidence that colonization of the caudal vagina and vestibule by bacteria is a sign of disease
- there is no evidence of coital influence on the vestibular bacterial flora
- withholding mating because of isolation of β-haemolytic streptococci from the vestibule seems unnecessary
- consideration should be given to the probability that the dog has a resident penile flora (see 17.2) which may be enhanced by contact between the penis and the bitch's coat, anus, etc, before intromission
- the isolation of a single species of bacterium (pure culture) from the tract may be more significant than a mixed culture

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- venereal spread of organisms other than bacteria is possible, but has not been confirmed
- much more research is necessary before the possible role of bacteria in bitch infertility can be elucidated
- the only known bacterial venereal pathogen in the dog is Brucella canis
- routine bacteriological screening in the clinically normal bitch is pointless

5.11 CYTOLOGICAL EXAMINATION OF SUSPECTED PATHOLOGICAL MATERIAL

- cytological examination may be useful for determining the cause of abnormal discharges, swellings and masses
- fine-needle aspirates, 'grab' biopsies collected endoscopically and excised material may be useful
- typical features of inflammatory disease include increased numbers of polymorphonuclear neutrophils and bacteria where these are involved
- typical features of neoplasia are specific to the lesion, for example:
  (a) vaginal leiomyomas have typical elongate spindle cells with cigar-shaped nuclei
  (b) transmissible venereal tumours (in countries where this is present) have round cells with eccentrically located nuclei and coarsely stippled chromatin
  (c) ovarian germ cell tumours contain round cells with nuclei containing finely stippled chromatin in the nuclei and a lightly basophilic cytoplasm

5.12 ENDOCRINOLOGICAL STIMULATION TESTS

- measurement of basal hormone concentrations is not always diagnostic
- hormone stimulation tests may be useful to confirm the presence of a functional pituitary – ovarian interaction (the gonadotrophin-releasing hormone (GnRH) stimulation test)
- hormone stimulation tests may be useful to confirm the presence of functional ovarian tissue (the human chorionic gonadotrophin (hCG) stimulation test)

The gonadotrophin-releasing hormone (GnRH) stimulation test (Figure 5.6)

- a plasma (serum) sample is collected and assayed for LH and oestrogen concentration

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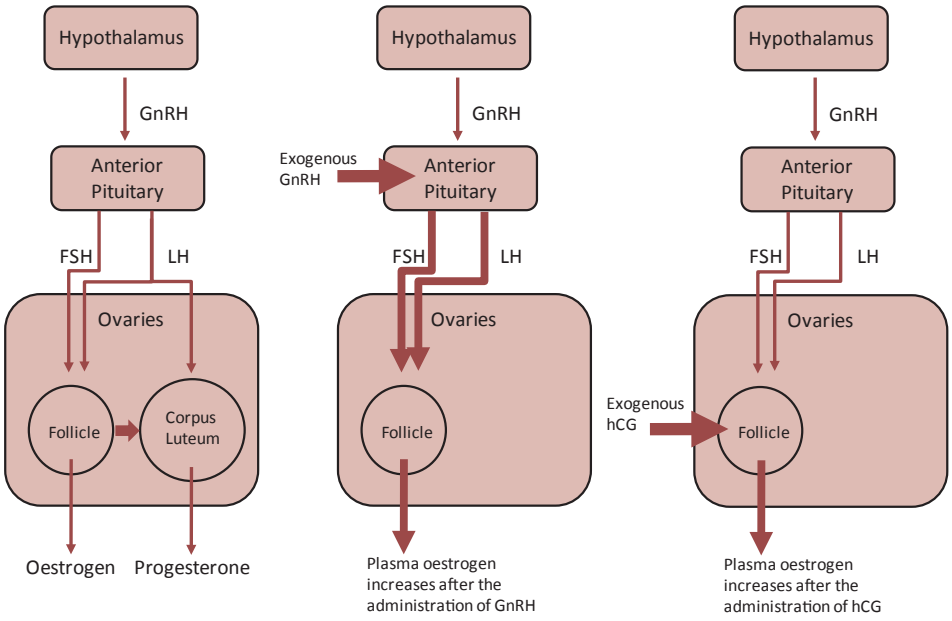


Figure 5.6 Injection of GnRH and hCG causes increased secretion of oestrogen in the normal bitch.

- a GnRH analogue is injected intravenously (0.01 mg/kg buserelin) and a second sample is assayed for LH and oestrogen concentration 60 minutes later
- a significant increase in LH concentration indicates the presence of a functional pituitary gland
- a significant increase in oestrogen concentration indicates the presence of a functional pituitary gland and ovary (the test may be used to confirm the presence or absence of ovarian tissue in bitches with an unknown reproductive history)

The human chorionic gonadotrophin (hCG) stimulation test (Figure 5.6)

- hCG is used rather than LH because the latter is not commercially available
- hCG is principally LH-like in activity
- a plasma (serum) sample is collected and assayed for oestrogen concentration
- hCG is injected intravenously (50 IU/kg) and a second sample is assayed for oestrogen concentration 60 minutes later
- a significant increase in oestrogen concentration indicates the presence of functional ovarian tissue (the test may be used to confirm the presence or absence of ovarian tissue in bitches with an unknown reproductive history)

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5.13 LAPAROSCOPY AND LAPAROTOMY

- laparoscopy and laparotomy are not widely performed for the investigation of reproductive tract disease
- both techniques may be useful when other diagnostic tools have failed
- these techniques enable abnormalities of the tubular genital tract to be diagnosed and for cytological specimens to be collected where appropriate

5.14 KARYOTYPING

- this is establishing the number and appearance of chromosomes
- may be useful in certain cases of infertility especially animals with ambiguous genitalia or bitches that never demonstrate cyclical activity
- there are a limited number of institutions offering this service
- requires collection of blood into an EDTA anticoagulant and then rapid transport to the laboratory
- chromosomes are examined from metaphase spreads following culture
- the most common abnormalities seen in the bitch where there is a phenotypic female appearance are XO syndrome and XX sex reversal

REFERENCES

1 Moxon, R., Copley, D. and England, G.C.W. (2010) Quality assurance of canine vaginal cytology: a preliminary study. *Theriogenology* **74**, 479–485.
2 England, G.C.W. (1992) Vaginal cytology and cervico vaginal mucus arborisation in the breeding management of bitches. *Journal of Small Animal Practice* **33**, 577–582.
3 England, G.C.W. and Allen, W.E. (1989) Crystallization patterns in anterior vaginal fluid from bitches in oestrus. *Journal of Reproduction and Fertility* **86**, 335–339.
4 England, G.C.W. and Yeager, A.E. (1993) Ultrasonographic appearance of the ovary and uterus of the bitch during oestrus, ovulation and early pregnancy. *Journal of Reproduction and Fertility* **47**(Supplement), 107–117.
5 England, G.C.W. and Allen, W.E. (1989) Real–time ultrasonic imaging of the canine ovary and uterus. *Journal of Reproduction and Fertility* **39**(Supplement), 91–100.
6 England, G.C.W. and Allen, W.E. (1989) The ultrasonographic and histological appearance of the canine ovary. *Veterinary Record* **125**, 555–556.
7 England, G.C.W., Russo, M. and Freeman, S.L. (2009) Follicular dynamics, ovulation and conception rates in bitches. *Reproduction in Domestic Animals* **44**, 53–58.
8 England, G.C.W., Allen, W.E. and Porter, D.J. (1989) A comparison of radioimmunoassay with quantitative and qualitative enzyme–linked immunoassay for plasma progestogen determination in bitches. *Veterinary Record* **125**, 107–108.
9 Moxon, R., Copley, D. and England, G.C.W. (2010) Technical and financial evaluation of assays for progesterone in canine practice in the UK. *Veterinary Record* **167**, 528–531.
10 England, G.C.W. (1991) ELISA determination of whole blood and plasma progestogen concentration in bitches. *Veterinary Record* **129**, 221–222.

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11 England, G.C.W. and Anderton, D.J. (1992) Determination of progestogen concentrations in the vaginal fluid of bitches in oestrus. *Veterinary Record* **130**, 143–144.
12 Jeffcoate, I.A. and England, G.C.W. (1997) Urinary LH, plasma LH and progesterone and their clinical correlates in the periovulatory period of domestic bitches. *Journal of Reproduction and Fertility* **51**(Supplement), 267–275.

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6 Anatomy of the Male

Understanding of the normal anatomy of the male reproductive tract is important to facilitate examination of the male for normality and to determine changes from normal which may result in disease; it is also helpful in some cases for determining the prognosis for fertility.

6.1 PREPUCE (SHEATH)

- completely covers the non-erect penis
- the outside is covered by skin
- the inside is a mucous membrane which is continuous with the mucosa of the penis at the base of the glans penis
- occasionally the prepuce is short in length in comparison to the penis which results in a protrusion of the penis

6.2 PENIS

- the glans penis starts at the attachment to the inner lining of the prepuce and is composed of:
  (a) the proximal bulbus glandis – in the non-erect state this is hardly discernible; when the penis is erect the bulbus glandis becomes a roughly spherical swelling which is responsible for locking the penis into the bitch's vagina during coitus (see 9.4)
  (b) the pars longa glandis comprises the distal three-quarters of the glans and terminates in the opening of the urethra
- the os penis is a bone which is surrounded by the glans penis; it is broad proximally and narrower distally and has a ventral groove which houses the urethra

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6.3 TESTES (Figure 6.1)

- male dogs are born with testes within the abdomen
- testes generally pass through the inguinal canal 4 days after birth
- the scrotal location is normally reached by 4–6 weeks after birth and usually earlier
- testes are roughly ovoid in shape with the long axis of the testis lying longitudinally
- the bulk of the testis is composed of seminiferous tubules, in which spermatozoa are produced by the process of spermatogenesis
- the seminiferous tubules are supported by a network of fibrous tissue called the mediastinum testis
- the seminiferous tubules empty into a collecting system, the rete testis, which conducts spermatozoa away from the testis and into the epididymis
- normal testes have a firm texture upon palpation; changes in texture, either local or generalized, softening or hardening, usually indicate disease
- there is a good relation between testicular volume and total sperm output; data from the author's clinic has established normal ranges that are now widely used in clinical practice [1]

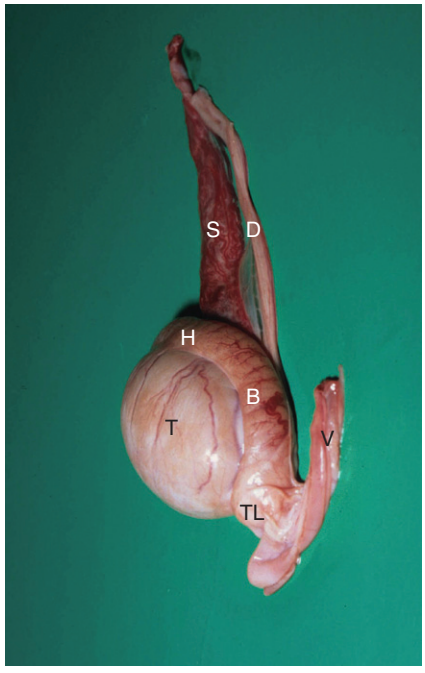


Figure 6.1 Testis and adnexa, lateral view: testis (T), head (H), body (B), and tail (TL) of epididymis, spermatic artery and vein (S) and ductus deferens (D). The tail of the epididymis is attached to the tunica vaginalis propria (V) by the ligament of the tail of the epididymis.

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6.4 EPIDIDYMIS

- this is a very long tube which is coiled tightly onto itself to form a structure which can be described as having a head, body and tail
- the head (caput) of the epididymis is on the cranio-lateral border of the testis and is not readily palpable
- the body (corpus) of the epididymis is on the dorso-lateral surface of the testis and cannot be palpated
- the tail (cauda) of the epididymis is on the dorso-caudal pole of the testis; it is readily palpable in the normal animal as a firm 'pea-sized' protuberance which does not appear to be contiguous with the body
- the epididymis now continues as a straight tube which contains mature spermatozoa and is called the ductus (vas) deferens
- during their passage through the epididymis spermatozoa become mature; in the dog this takes about 14 days
- in dogs with an absence of sperm production the tail of the epididymis may be smaller than the normal size and have a flaccid texture upon palpation

6.5 DUCTUS DEFERENS (VAS DEFERENS)

- this duct conveys spermatozoa from the epididymis to the urethra
- it runs into the abdomen in the spermatic cord through the inguinal canal
- within the abdomen it passes cranial to the ureter and then empties into the urethra in the cranial prostate gland
- the ducti (vasa) deferentia are approximately 1–2 mm in diameter, pale coloured and have a firm texture – they are good landmarks for locating abdominal testes during surgery

6.6 SPERMATIC CORD

- this is a bundle of various tissues which runs between the testis and the abdominal wall
- it has four main components:
  (a) the ductus deferens
  (b) the cremaster muscle: this arises from the internal abdominal oblique muscle near the external inguinal ring and is attached to the vaginal tunic at the level of the testis; it can vary the distance between the testis and abdominal wall, thus regulating testis temperature
  (c) the spermatic (testicular) artery: this conveys blood from the aorta to the testis and runs through the pampiniform plexus
  (d) the spermatic (testicular) vein: this conducts blood from the testis to the vena cava; in the spermatic cord the vein divides into a complex of small veins (the pampiniform plexus) which surrounds the spermatic artery. The cool blood returning from the testicle reduces the temperature of the arterial blood to ensure that the testis does not become overheated

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NB: A special muscle in the scrotal skin, the dartos, can also regulate the proximity of the testes to the body wall and thus influence their temperature.

6.7 INGUINAL CANAL

- the spermatic vessels and the ductus deferens pass into the abdomen through a narrow space in the muscles of the abdominal wall – the inguinal canal
- occasionally this canal is wide enough to allow the escape of some abdominal contents to form an inguinal hernia; this may also happen in the bitch (see 21.7)
- close to the inguinal canal is the inguinal lymph node that may become enlarged when there is testicular pathology

6.8 PROSTATE GLAND

- this is the only accessory gland in the male dog
- it is a bi-lobed structure situated at the pelvic inlet (Figure 6.2)

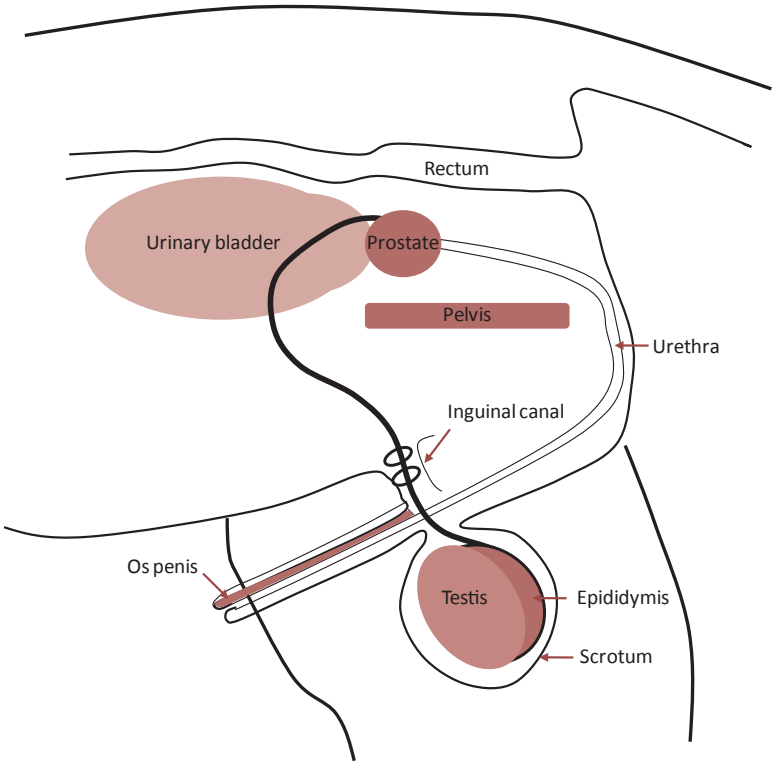


Figure 6.2 Schematic representation of the anatomy of the reproductive tract of the dog.

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- the urethra passes through the gland before it reaches the base of the penis
- the ducti (vasa) deferentia also pass through a small amount of the cranial aspect of the gland before they enter the urethra dorsally
- the prostate gland produces a clear secretion which is expelled into the urethra
- studies in the author's laboratory have confirmed that the prostate gland contributes the first and third fractions of the ejaculate [2]
- prostatic fluid is bacteriocidal
- the position and size of the gland are very variable
- the gland normally increases in size with advancing age; increase in size is usually accompanied by a more abdominal position
- the gland is prone to pathological changes which also cause an increase in size
- a range of diagnostic tests can be used to differentiate normal from abnormal prostate (see 17.7)

6.9 URETHRA

- this tube has the function of conveying both urine and semen to the tip of the penis
- it originates at the bladder neck and travels caudally on the floor of the pelvis through the prostate gland; here the ducti deferentia open into its dorsal surface
- at the caudal limit of the ischium the urethra enters the penis and runs on its caudo-ventral aspect eventually entering the ventral groove of the os penis
- coordinated muscular activity is required for the forceful emission of semen

REFERENCES

1 England, G.C.W. (1991) The relationship between ultrasonographic appearance, testicular size, spermatozoal output and testicular lesions in the dog. *Journal of Small Animal Practice* **32**, 306–311.

2 England, G.C.W., Allen, W.E. and Middleton, D.J. (1990)An investigation into the origin of the first fraction of the canine ejaculate. *Research in Veterinary Science* **49**, 66–70.

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7 Physiology of the Male

There is complex regulation of reproductive physiology, with a close association between the hormonal environment and the reproductive organs. This is necessary to provide suitable male behaviour and sperm production.

7.1 SPERMATOGENESIS

- this is the complicated process by which spermatozoa are produced, and it occurs in the seminiferous tubules of the testes
- cells called spermatogonia, the precursors of spermatozoa, divide in the usual manner (mitosis) to produce very many spermatocytes
- spermatocytes then divide by meiosis in which the normal number of chromosomes in the resulting cells is halved to 39 and the genetic material derived from the individual's parents is mixed
- the resulting cells are called spermatids and are described as being haploid (half the usual number of chromosomes – cells with 78 chromosomes are said to be diploid; this includes the two sex chromosomes)
- spermatids change into spermatozoa by a complex rearrangement of organelles; basically the nucleus becomes the sperm head, the Golgi apparatus forms the acrosome, and the mitochondria and centrioles are involved in the development of the mid piece and tail (see 10.5)
- most of the cytoplasm is left in the Sertoli cells which are situated on the basement membrane of the seminiferous tubule; they regulate the metamorphosis from spermatid to spermatozoon
- some components of the cytoplasma remain attached to sperm as 'residual bodies' – these are frequently seen in (immature) ejaculated spermatozoa as distal cytoplasmic droplets; proximal cytoplasmic droplets are less commonly seen and represent very immature sperm that are incapable of fertilization
- spermatogenesis commences at approximately 4 months of age but spermatozoa do not appear in the ejaculate until 10–12 months on average
- we have shown interesting heritable traits relating to semen quality in dogs; these studies suggest that breeding selection procedures may be useful in an attempt to improve semen quality within a breeding programme [1]

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7.2 TEMPERATURE CONTROL

- spermatogenesis cannot occur at normal body temperature in most mammals
- the mechanisms which keep the dog's testes cooler than the rest of the body have been mentioned in Chapter 6 and are:
  (a) the testes are housed outside the body in the scrotal sac
  (b) the cremaster muscle can influence the distance between the body and testis
  (c) the dartos muscle in the scrotal wall can influence the size of the scrotum and thus the position of the testes
  (d) the arrangement of the blood vessels in the spermatic cord allows cooling of arterial blood by the venous return in the pampiniform plexus (see 6.6)
- conditions which cause elevated body temperature, e.g. fever and heat stroke, may not be compensated for by these mechanisms and can impair spermatogenesis (sometimes on a permanent basis)
- obesity may also reduce testicular cooling

7.3 SPERMATOZOAL TRANSPORT

- seminiferous tubules are 'U'-shaped and along the tubule all the different stages of spermatogenesis are represented; sperm are therefore continually released into the lumen (this would not be the case if all areas of the tubule had spermatogenesis occurring at the same stage of development)
- luminal sperm enter collecting ducts and then enter the caput of the epididymis
- spermatozoa entering the epididymis are in an immature state; they have a droplet of residual cytoplasm at the neck called a proximal cytoplasmic drop (see 10.5) which is sometimes incorrectly called the 'protoplasmic' droplet
- during the passage of spermatozoa along the convoluted epididymis, which is presumably caused by the constant production of more spermatozoa from the testis, the cells mature
- on entry into the ductus deferens (the epididymal transit time is 14 days in the dog) the cytoplasmic droplet has moved to the distal end of the mid piece or become detached from the spermatozoon, which is now considered to be mature
- factors which might influence the speed of movement along the ductus deferens are unknown
- sperm are generally considered to be stored within the tail of the epididymis; in the normal dog the pea-sized tail is normally turgid and quite plump upon palpation but small and flaccid where sperm production is low
- spermatozoa which are not voided by ejaculation are thought to be forced to the opening of the ductus deferens and into the urethra by the build-up of cells behind them; they may then travel cranially to the bladder and are expelled in urine

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- during ejaculation spermatozoa are actively expelled from the ductus deferens into the urethra
- there is little evidence to support the concept that the ejaculate from an infrequently 'used' stud dog will contain an abnormally high number of abnormal spermatozoa
- the spermatogenic cycle, i.e. from division of spermatogonia to the appearance of spermatozoa in the ejaculate, takes 8 weeks; for 2 of these weeks the spermatozoa are maturing in the epididymis

7.4 PROSTATE GLAND

- produces the first and third fractions of the dog's ejaculate (see 10.5) [2]
- the function of the first fraction is probably to flush the urethra clear of any urine or cellular debris
- incubation of second rich fraction with first fraction in a water bath is detrimental to sperm; in normal mating the first fraction is expelled during play before intromission and therefore does not enter the bitch
- the function of the third fraction of the ejaculate is probably to wash the sperm-rich second fraction from the cranial vagina into the uterus of the bitch
- disease of the prostate (17.7) may affect fertility because:
  (a) blood can affect spermatozoal viability in some cases but this is unlikely in dogs
  (b) sperm often stick to polymorphonuclear neutrophils (PMNs) and therefore do not progress in the female tract
  (c) the effect of bacterial prostatic infection (and subsequent changes in the nature of the prostatic fluid) on spermatozoal function is unknown
  (d) drugs used to reduce prostatic size (progestogens; gonadotrophin releasing-hormone agonists) may also inhibit spermatogenesis (see 27.1 and 27.5); other drugs such as alpha-reductase inhibitors (which essentially are anti-androgenic [see 27.11]) reduce the volume of prostatic fluid ejaculated resulting is a small volume but concentrated ejaculate
- surprisingly, spermatozoa incubated in vitro in prostatic fluid are less viable than those incubated in semen extenders; it seems therefore that prostatic fluid per se is not supportive of spermatozoal function
- prostatic fluid is probably important for preventing the attachment of sperm to PMNs and for modifying specific components of the uterine inflammatory response to mating

7.5 ERECTION

- factors which stimulate erection in most animals are pheromones, the smell of a female in oestrus and the association between routine and coitus
- the pathways by which these stimuli initiate erection are probably neural, but are not completely understood

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- erection is tumescence (swelling and turgidity) of the penis due to an accumulation of blood in its tissues caused by constriction of venous return
- in dogs only slight erection occurs before coitus; intromission is facilitated by the rigidity afforded by the os penis
- during full erection there is substantial swelling of the bulbus glandis which results in the penis becoming 'locked' into the female genital tract (see 9.4)
- it is only after the erection has subsided that the dog and bitch can subsequently part

7.6 EJACULATION

- contractions of the urethral muscle force fluids from the ducti deferentia and prostate gland into and along the urethra
- there are three distinct fractions of the ejaculate that can be identified

First fraction

- during initial sexual excitement the first fraction of the ejaculate is voided
- this varies in volume but is usually approximately 0.5–2.0 ml
- this fraction may also be ejaculated while the dog is thrusting during attempts to introduce his penis into the bitch's vagina, but is not usually voided during intromission
- work in the author's laboratory demonstrated that this fraction originates from the prostate gland and may have the function of washing the urethra clear of urine [2]

Second fraction

- the second fraction is usually ejaculated after intromission when thrusting ceases
- this is the sperm-rich portion of the ejaculate and is usually 0.5–1.0 ml in volume
- it originates from the ducti deferentia and is deposited into the cranial half of the vagina during completion of the dog's erection
- during and soon after ejaculation of this fraction, the dog instinctively wants to 'turn' (see 9.4)

Third fraction

- the third fraction originates from the prostate gland
- it is usually voided whilst the dog and the bitch are standing tail to tail in the 'tie' position (Figure 7.1)

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Figure 7.1 Ejaculation of the third (prostatic) fraction of the ejaculate occurs during the 'tie'.

- the volume of prostatic fluid ejaculated may approach 15–20 ml in large breeds and is probably related to the length of the 'tie'
- the function of this fraction is thought to be that of washing the spermatozoa forward into the uterus; however, the effect of third fraction on spermatozoa may not always be favourable (see 7.4)
- termination of ejaculation is followed by detumescence of the penis and its withdrawal
- normally after ejaculation the dog licks his penis until it returns to its position within the prepuce

7.7 SPERM TRANSPORT IN THE BITCH

- in the bitch the male accessory gland secretions have critical importance
- we have shown that the bitch has a uterine inflammatory response to sperm deposition, but both seminal plasma and prostatic fluid have a modulatory effect on this, most likely mediated by a reduction in neutrophil binding to sperm [3]

REFERENCES

1 England G.C.W., Phillips, L. and Freeman, S.L. (2010) Heritability of semen charcteristics in dogs. *Theriogenology* **15**,1136–1140.

2 England, G.C.W., Allen, W.E. and Middleton, D.J. (1990) An investigation into the origin of the first fraction of the canine ejaculate. *Research in Veterinary Science* **49**, 66–70.

3 England, G.C.W., Russo, M. and Freeman, S.L. (2012) The bitch uterine inflammatory response to mating and its modification by male accessory gland secretion. *The Veterinary Journal* (in press).

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8 Endocrinology of the Male

The same hormonal axis is present in the male dog as seen in the bitch, i.e. secretion from the hypothalamus stimulates the pituitary gland; secretion from the pituitary gland stimulates the testes (Figure 8.1) [1].

8.1 GONADOTROPHIN-RELEASING HORMONE (GnRH)

- produced in the hypothalamus (at the base of the brain) and conveyed to the anterior pituitary gland via a specialized system of blood vessels
- responsible for selectively causing release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) from the anterior pituitary gland
- secretion is episodic
- pulsatile secretion rather than continuous secretion is necessary for the induction and maintenance of normal gonadotrophin release

8.2 FOLLICLE-STIMULATING HORMONE (FSH)

- FSH release is slow and gradual after GnRH stimulation
- FSH binds to Sertoli cells and spermatogonia
- binding results in the production of a variety of proteins that may be important in regulating spermatogenesis via androgen binding protein and transferrin
- feedback control of FSH is via the gonadal peptide inhibin, and possibly modulation of Leydig cell function

8.3 LUTEINIZING HORMONE (LH) (ALSO CALLED INTERSTITIAL CELL STIMULATING HORMONE (ICSH))

- LH release is immediate but transient after GnRH stimulation
- plasma concentrations increase and return to basal within 60 minutes
- LH binds to Leydig cells of the testis

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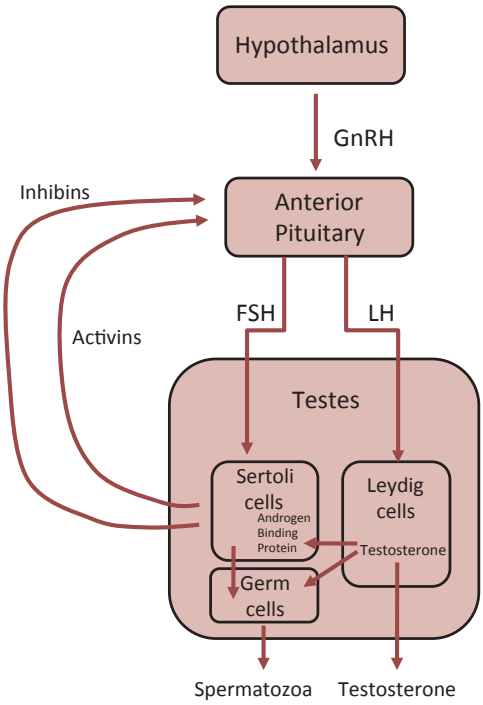


Figure 8.1 Schematic representation of hormone production in the male dog.

- LH stimulates testicular steroidogenesis
- a rise in plasma LH is followed by a rise of plasma testosterone within 60 minutes

8.4 ANDROGENS (TESTOSTERONE AND DIHYDROTESTOSTERONE)

- produced by cells in the testis which form little islands between the seminiferous tubules; these are interstitial cells or Leydig cells
- testosterone secretion occurs both locally within the testis and into the peripheral circulation
- most testosterone secreted into the plasma is bound to plasma proteins
- peripheral testosterone is necessary for the development and maintenance of secondary sexual characteristics, maintenance of sexual behaviour and negative feedback regulation of gonadotrophin secretion
- high intra-testicular testosterone concentrations are maintained within the seminiferous tubules by binding to androgen binding protein (ABP)

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- high local testosterone concentrations are important for the initiation and maintenance of spermatogenesis
- testosterone and its active metabolites oestradiol and dihydrotestosterone exert a profound feedback effect on FSH and LH
- ectopic testes (e.g. in cryptorchid dogs; see 17.3) still produce androgens and have male physical characteristics and behaviours
- in mature dogs plasma testosterone concentrations vary between 0.5 and 5.0 ng/ml; there is a great deal of variation throughout the day in an individual dog, so that assay of a single sample is of little value [2]
- in castrated dogs, values are consistently below 200 pg/ml
- in order to diagnose the presence of an unidentified ectopic testis a human chorionic gonadotrophin (hCG) stimulation test can be performed (see 10.15); a rise in testosterone concentration after administration of hCG indicates the presence of testicular tissue [2]

8.5 INHIBIN

- this is a hormone produced by the Sertoli cells of the testis which inhibits the release of FSH
- its physiology in the dog has not been proven but it is assumed based on information in other species

8.6 ACTIVINS AND INHIBINS

- there is increasing evidence that the Sertoli cells produce other substances, termed activins that have a stimulatory effect upon FSH secretion

REFERENCES

1 England, G.C.W. (1994) Reproductive endocrinology in the dog and bitch. *Journal of Postgraduate Clinical Study In Practice* **16**, 275–279.

2 England, G.C.W., Allen, W.E. and Porter, D.J. (1989) The evaluation of the testosterone response to human chorionic gonadotrophin, and the identification of a presumed anorchid dog. *Journal of Small Animal Practice* **30**, 441–443.

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9 Normal Mating

9.1 PLANNING THE MATING

- critical for planning a mating is appropriate research and planning ahead
- selection of which animals to breed is a complicated art and much is detailed in sections 1.5 and 1.6
- before breeding it is important to consider whether there are adequate time and finances to support the bitch especially if something should go wrong and for example a caesarean operation is needed
- recognizing whether there is an appropriate market for the pups that will be produced is essential
- there is no physiological or medical reason why a bitch must have a litter of pups; indeed it may be in the best interest of the individual for her to be neutered
- breeders may be required to have a license for this purpose (for example in the UK the Breeding of Dogs Act 1973 legislates about licensing and governs the conditions under which animals should be managed and how frequently they can be bred)
- when breeding involves animals owned by different people it is best to use a breeding contract which should contain details of the stud fee, whether it is payable if the bitch does not conceive, whether the bitch has a free return if she does not conceive and whether the stud dog owner has the 'pick' of the litter of pups
- the help of an experienced dog breeder should be sought if the bitch and dog owner are novices
- inform the dog owner when the first signs of pro-oestrus are noted in the bitch

9.2 TIMING

- most bitches are mated 10–12 days after the beginning of pro-oestrus
- this usually gives satisfactory results even if the bitch is not ovulating at this time because:
  (a) sperm from a fertile dog can survive for up to 7 days in the bitch, so that mating before ovulation can be fertile

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(b) oocytes are not ready to be fertilized until 2 days after ovulation, so that mating after ovulation may be fertile (see 11.3)

- however, an inappropriate mating time is the most common cause of alleged infertility in the bitch (see 11.1)
- repeated mating 24–48 hours apart increases the likelihood of conception
- help in detecting ovulation time may be required when:
  (a) bitches ovulate much earlier or later than average (see 3.4)
  (b) artificial insemination is being used (see 27.1)
  (c) a dog with poor semen quality is being used; repeated mating may still be advantageous (see 17.3)
  (d) a bitch is 'shy' and may need to be restrained during mating
- ovulation time can be detected using vaginal endoscopy, vaginal cytology, or by measuring blood progesterone concentrations (see 11.3)

9.3 PREPARATION FOR MATING

- the appropriate stud to be used should be identified long before the onset of pro-oestrus to enable careful checking of health status information (hip scores etc.)
- vaccination and disease status of individual and the kennel to be visited should be examined
- despite what is expected by many breeders there is no point in routine bacteriological screening of the male or female unless in a country where Brucella canis is important
- it is common practice for the female to visit the male
- immediately before mating it is helpful to clip long hair from around the vulva; the vulva may also be cleaned with water to reduce bacterial contamination which may be flushed into the uterus at the time of mating
- the bitch should be encouraged to urinate before being introduced to the dog
- both dog and bitch should be restrained using a long lead for control, but allowing play including mounting before intromission
- oil-based lubricants are placed on the vulval lips by some breeders especially when inexperienced animals are mated; whilst not necessary these are helpful

NB: Oil-based lubricants are preferable to water-based lubricants which can mix with the sperm and have a toxic effect.

9.4 NORMAL COITAL BEHAVIOUR

Foreplay

- if dog and bitch are unrestrained a variable amount of sniffing, jumping and playing occurs, during which time the dog, particularly if he is inexperienced, will mount the bitch in various inappropriate positions

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- during this time first fraction may be ejaculated
- if the bitch is cooperative she will eventually stand still to allow the dog to mount her from behind, and she will raise her tail to one side and lift her vulva to facilitate intromission
- some bitches may need to be held by the head and be muzzled in order to prevent the risk of the dogs biting each other, or an aggressive bitch frightening an inexperienced dog

Intromission

- once the dog has mounted he will commence thrusting movements in order to try to achieve intromission; at this time erection is only slight and rigidity is afforded by the os penis (see 6.2)
- the fore limbs of the dog grip the bitch tightly in front of her pelvis
- on gaining intromission more purposeful thrusts ensure that the whole glans penis is inserted; the dog's pelvis is forced very close to the bitch at this stage, and his lower back is almost vertical
- erection starts after full intromission, and the dog ejaculates the second, sperm-rich fraction at this time

The 'tie'

- once full erection has occurred the dog instinctively wants to dismount
- by placing both front feet to one side of the bitch, the back leg on the opposite side is left in the air
- the dog lifts this leg over the bitch's back whilst turning round
- this causes the penis, proximal to the os penis, to bend laterally through 180 degrees
- if the dog has a full erection, his penis will not leave the bitch's vagina
- this twisting is not painful and probably functions to maintain blood within the penis and prolong the ejection and lengthen the time of ejaculation
- ejaculation of the third fraction continues, even though the urethra is bent

NB: In some breeds, e.g. greyhounds, a complete turn is not usually allowed by the handler; this does not seem to affect fertility, but is an inappropriate strategy.

Practical management of breeding

- the dog must be able to enter the bitch as straight as possible and this could be difficult if he is too short or too tall; a step or sloped surface can be used for either the dog or bitch to address size differences

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- sometimes the dog will not breed because he has had an unpleasant past experience that has resulted in a loss of confidence, often causing a failure to achieve a full erection; holding the dog and bitch together as soon as thrusting has stopped may be helpful
- a dog should not be allowed to mate the bitch too many times without achieving a tie; it is better to try a few times and then rest the dog until the next day
- often the bitch's position can be a problem: she may not elevate her vulva correctly, she may not deviate her tail very well, or she may keep moving her tail from side to side; in these instances elevating the bitch's vulva to the correct position or holding her tail out of the way can help the dog
- maiden bitches (bitches that have not been mated before) can be a little overwhelmed by the whole process and require much more support than an experienced bitch; they will often stand at first but then change their minds. In these cases patience and gentle encouragement is needed; sometimes these matings take several hours to achieve
- some bitches can be difficult if the stud dog is playing too much and leaping on the bitch; inexperienced dogs exhibit this type of behaviour. The problem is rectified by gentle restraint of the bitch, ensuring that the human presence does not upset the stud
- whenever possible an inexperienced stud dog should be put with an experienced bitch, and a new bitch with an experienced stud

9.5 FACTORS PREVENTING NORMAL MATING
(see 20.6 and Chapter 24)

Psychological factors

- Most dogs know instinctively how to copulate but on occasion may not be able to because of:
  (a) a difference in sexual experience; an experienced stud may frighten a virgin bitch or vice versa
  (b) fear of human disapproval; this is particularly true of male dogs which have previously been reprimanded for displaying sexual interest
  (c) unfamiliarity with the venue; because the dog takes the more active role in coitus the bitch is usually taken to the dog's residence in order to avoid this problem
  (d) overfamiliarity; sometimes dogs and bitches kept together all the time will not copulate
  (e) humanization: particularly bitches, when kept in houses and rarely exposed to other dogs, appear to resent the approach of another dog as if it were 'abnormal'
  (f) lack of libido: in male dogs this may be similar to the previously mentioned problem in the bitch. The cause could also be some of the other aforementioned psychological factors

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- diagnosis and therapy are difficult:
  (a) measurement of circulating testosterone concentrations is not diagnostic because of the large variation throughout the day in the normal dog
  (b) administration of human chorionic gonadotrophin (hCG) may increase testosterone production but is not therapeutic
  (c) administration of testosterone is also not therapeutic and may cause aggression and impaired spermatogenesis (due to inhibition of release of pituitary gonadotrophins)
  (d) thyroid replacement therapy may improve libido but such regimes have not been properly evaluated

Physical factors

- dogs with obvious limb deformities and musculo-skeletal lesions may have problems achieving a position compatible with intromission; they should not be used for breeding if their condition is inherited
- subtle lumbar or lumbo-sacral lesions may prevent dogs from mating, even though there is no evidence of pain in other everyday activities
- bitches may require help to hold up heavy dogs
- dogs with prostatic disease may show signs of pain during or after ejaculation (see 17.7)
- non-steroidal anti-inflammatory drugs may reduce musculo-skeletal pain and are not known to be detrimental to semen

NB: It may be difficult to ascertain whether a dog's reluctance to copulate is due to physical or psychological factors.

Failure to tie

- after intromission the tie may not occur due to both dog and bitch factors (commonly size difference)
- if ejaculation of the second fraction has occurred, the bitch may still conceive, although fertility is not thought to be good presumably because of the absence of flushing sperm forward within the vagina into the uterus
- in the author's laboratory we have documented the critical effect that dog prostatic fluid has in modulating the bitch uterine inflammatory response to mating [1,2]; failure to tie may also result in lower fertility since prostatic fluid is not present to ameliorate the uterine inflammatory response to sperm
- the effect of the length of the tie on fertility is not known, although many breeders feel that a long tie enhances the chance of conception
- where the dog persistently separates quickly some breeders will attempt holding the male and female together; the efficacy of this is unknown

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9.6 CERTIFICATE OF MATING

- in some countries it is necessary to for the dog and bitch owner to jointly certify that natural mating occurred; specific nationally approved certificates are available for this purpose
- in other countries certification is only required at the time of registration of the pups

REFERENCES

1 England, G.C.W., Russo, M. and Freeman, S.L. (2012) The bitch uterine inflammatory response to mating and its modification by male accessory gland secretion. *The Veterinary Journal* (in press).

2 England, G.C.W., Moxon, R. and Freeman, S.L. (2012) Stimulation of mating-induced uterine contractions in the bitch and their modification and enhancement of fertility by prostatic fluid. *Reproduction in Domestic Animals* (in press).

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10 Clinical Examination of the Male

A careful clinical examination of the male can be helpful to document normality and in some cases the diagnosis and prognosis of a problem.

10.1 BASIC EXAMINATION OF THE REPRODUCTIVE TRACT

This consists of:

- visual examination of the prepuce and penis
- palpation of the scrotum
- semen collection and evaluation
- (measurement of alkaline phosphatase concentrations in semen)
- real-time ultrasonographic imaging
- measurement of plasma hormone concentrations
- observation of mating behaviour

10.2 ADDITIONAL TECHNIQUES THAT MAY BE CONSIDERED

These include:

- bacterial examination of the reproductive tract
- testicular biopsy
- testicular fine-needle aspiration
- prostatic biopsy
- prostatic massage/urethral flushing
- endocrinological stimulation tests
- karyotyping

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10.3 VISUAL EXAMINATION OF THE PENIS AND PREPUCE

- it is best initially to examine the dog in the absence of the bitch otherwise he may be difficult to handle
- a mucopurulent discharge from the prepuce is common and normal
- the prepuce should cover the length of the penis
- the os penis can be palpated through the prepuce and penis and it should be smooth in shape and not painful
- the penis can normally be relatively easily pushed out of the prepuce to enable it to be examined; in experienced stud dogs this may initiate ejaculation, which should be anticipated so that semen can be collected if this occurs
- multiple lymphoid nodules are frequently found at the base of the penis on the bulbus glandis; sometimes these may be traumatized at mating, by exteriorizing the penis, or during semen collection
- examination should also be performed on the erect penis after semen collection

10.4 PALPATION OF THE SCROTAL CONTENTS

- the scrotal skin should have no lesions; thickened skin or evidence of scarring is often associated with poor semen quality as there have been a previous scrotal (testicular) insult
- the testes should be freely mobile within the scrotum
- testes should be similarly sized and have reasonable tone
- the tail of the epididymides should be carefully palpated and should be firm in texture

10.5 SEMEN COLLECTION AND EVALUATION [1]

Reasons for semen evaluation

- to confirm normal spermatogenesis in a young dog before starting a stud career
- to monitor semen quality during the career of a stud dog
- to ensure suitable semen quality in an aged dog
- as part of any artificial insemination programme
- prior to semen preservation
- in cases of infertility where the dog may be implicated
- to monitor semen production after illness or drug therapy
- to investigate the effect of prostatic disease on semen quality

Semen collection

Collection prerequisites

- have a bitch in late pro-oestrus or oestrus present as this will help the procedure; some experienced dogs will ejaculate in the absence of a teaser, but if no

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sample is produced there is often then a question of whether this is a pathological process or lack of sexual excitement
- inexperienced dogs are unlikely to ejaculate in the absence of a bitch in oestrus
- the dog is usually taken to a venue strange to him where he is not used to 'working', which may inhibit even an experienced dog
- slippery floors or personnel wearing white coats may 'put the dog off'
- failure to collect a sample may upset the dog (and cause extra expense)

NB: Vaginal secretion on swabs from oestrous bitches may be stored frozen and used to stimulate otherwise reluctant dogs – the thawed swab is held in front of the dog's nose. The dog pheromone methyl-p-hydoxybenzoate is available commercially in the USA.

It is feasible to collect multiple samples from a fertile dog within 1 day [2].

Select an appropriate collection procedure

- owners or handlers who are normally present when the dog mates may encourage interest
- small breeds are often mated on a table with a suitable surface
- large breeds mate on the floor with various degrees of human guidance
- too many observers may distract the dog
- the ejaculate consists of three distinct fractions which, for proper evaluation, should be collected separately:
  (a) first fraction: prostatic fluid
  (b) second fraction: sperm-rich fluid
  (c) third fraction: prostatic fluid
- techniques for collection should therefore enable the fractional nature to be recognized and collected

Equipment

It is important that the equipment is not toxic to sperm; research in the author's laboratory has demonstrated the impact of collection equipment and a variety of processing steps on sperm viability; without recognizing these effects erroneous interpretation of fertility can be made [3,4].

- equipment required includes:
  (a) two or three glass or plastic funnels
  (b) two or three glass or plastic test tubes

NB: Plastic will not break, but because it is light in weight it can easily be dislodged from the hand by the dog's tail or leg.

  (c) a water bath, preferably maintained automatically at 37°C
  (d) a test tube rack in the water bath

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(e) microscope slides and cover slips
(f) ideally, a 'warm stage' which keeps a microscope slide at 37°C (a flat bottle filled with warm water may be used to stand the microscope slides on to keep them warm)
(g) pipettes
(h) nigrosin – eosin stain
(i) microscope with oil for high-power viewing
(j) Neubauer cell counting chamber
(k) equipment for 1/200 dilution of semen

Collection technique (Figure 10.1)

- the bitch is adequately restrained by the head or neck
- the dog is allowed to sniff and lick at the bitch's vulva
- when the dog seems interested, and before he tries to mount, the penis is held (usually by the operator's right hand, from the dog's left side)
- if the bulbus glandis is swollen, the prepuce should be pushed backwards to expose the whole glans
- if the bulbus glandis is not swollen, to and fro movement of the prepuce should stimulate the initial erection



Figure 10.1 Semen collection: (a) the prepuce is pushed backwards to expose the penis; (b) the penis is reflected backwards between the dog's legs; (c) continued pressure or rhythmic squeezing of the base of the bulbus glandis stimulates further erection to occur; (d) the penis is now fully erect and the ejaculate is being collected via a glass funnel.

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- if the bulbus glandis becomes too swollen it is not possible to protrude the glans penis from the sheath; the dog should be taken away from the bitch until his erection has subsided, or an attempt may be made to collect with the penis in the sheath – this may be uncomfortable for the dog
- after exposure of the bulbus glandis, the base of the glans penis is gripped between the thumb and fore finger; it may be held firmly or rhythmic contractions applied
- pressure on the base of the penis should stimulate further swelling of the bulbus glandis with or without thrusting movements of the dog
- apart from premature swelling of the bulbus glandis, a major problem in exposing the penis is in long-haired breeds, and in small breeds where holding the penis is difficult
- during violent thrusting movements there is no point in trying to collect the ejaculate because it will be the sperm-free first fraction
- if first fraction is collected, it will be seen to be clear fluid
- after expulsion of the first fraction the second (sperm-rich and white) fraction is ejaculated in 4–10 urethral contractions; if possible this should be collected separately in the second test tube
- occasionally the volume of first fraction can approach 5 ml; if a nervous dog produces no sperm-rich fraction after this, a further collection should be considered
- after the sperm-rich fraction has been expelled, there may be several unproductive urethral contractions until the third fraction is ejaculated
- if the second fraction is very concentrated and of small volume, several 'spurts' of third fraction may be necessary to wash it from the collecting funnel and into the test tube
- the majority of the third fraction is either collected into the tube containing first fraction, or the dog is left to finish ejaculating onto the floor

Alternative collection techniques

Latex cone

- the test tube may be attached to a latex cone, which is placed over the dog's penis; this is not ideal because it is difficult to collect the fractions separately and because the latex may be toxic to sperm

Artificial vagina

- this is essentially a cylindrical tube filled with warm water; it is inappropriate because:
  (a) it is unnecessary
  (b) it is complicated and cumbersome to use
  (c) it encourages prolonged contact between sperm and the latex liner which may cause complete immotility of the sperm

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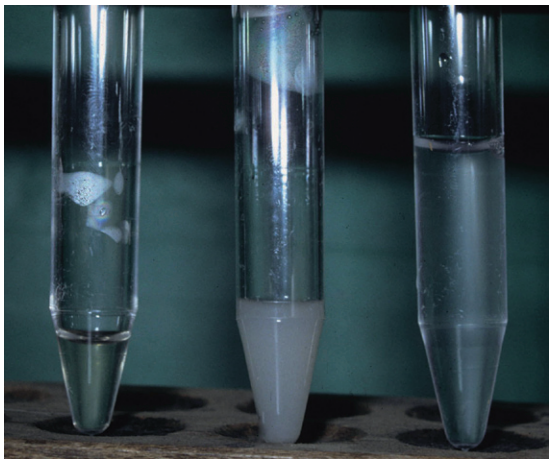


Figure 10.2 The three fractions of the ejaculate. The second fraction is opaque and contains the spermatozoa.

Evaluation of the sample (Figure 10.2)

- the sample must be kept warm because:
  (a) cooling of the sample causes a reduction in motility which will give a false impression of the quality of the sample
  (b) a rapid decrease in temperature may cause 'cold-shock' damage to the spermatozoa; this rarely occurs in dog semen as it is relatively resistant to cold shock, and semen is usually collected at room temperature

Volume of the ejaculate

- this is measured in a graduated test tube, or by comparing such a tube with the sample tube
- the first fraction is normally 0.5–2.0 ml in volume
- the second fraction is normally 0.5–1.0 ml in volume
- the third fraction is normally 15–20 ml in volume, but frequently this entire fraction is not collected

Colour of the ejaculate

- the first fraction is normally clear but may be contaminated with urine or cellular debris from the urethra
- the second fraction is normally white or creamy in colour
- the third fraction is normally clear
- occasionally there may be contamination with blood (especially the third fraction in middle-aged or old dogs), or other cells which will cause discoloration. If the sample is discoloured, examination of stained semen smears (e.g. using

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the modified Wright–Giemsa stain, Diff-Quik) may allow identification of contaminating cellular material such as red blood cells, or white blood cells

Spermatozoal concentration

This can be measured using:

- an electronic counting chamber which has been calibrated to count cells of this size. This is often inaccurate because sperm tails may lodge across the orifice of the device
- a colorimeter previously calibrated for dog semen. This has obvious inaccuracies
- a haemocytometer counting chamber (Figure 10.3), after suitable dilution of the sample:
  (a) a proportion of the semen is well mixed and is diluted 1 in 200 with distilled water containing a little detergent (the former kills spermatozoa and the latter prevents their clumping together)
  (b) one drop is placed into the chamber which has a standard depth and a known grid engraved upon its surface. Counting the number of spermatozoa within the grid (known area therefore known volume) allows calculation of the original spermatozoal concentration. It is customary to count squares diagonally across the grid. Normal values are between 300×10^6 and 800×10^6/ml, but the total spermatozoal output is a more useful measure than concentration alone

Total spermatozoal output

- this is a more meaningful measure than concentration or volume alone
- normal dogs produce between 300×10^6 and 1000×10^6 spermatozoa within each ejaculate

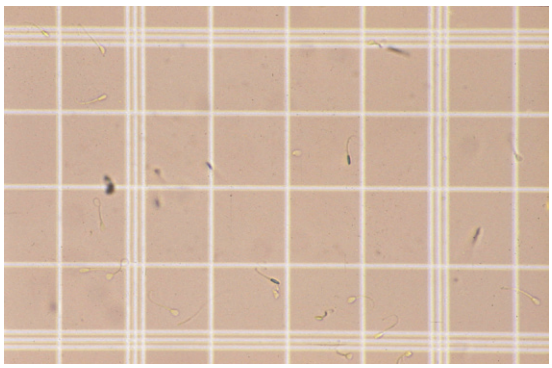


Figure 10.3 Counting chamber grid. Spermatoza are counted in five of the squares bounded by three parallel lines.

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Percentage motility

- the vessel containing the semen must be placed immediately into a water bath at approximately 37 °C to prevent cooling. A drop of semen is then placed onto a warmed microscope slide and covered with a cover slip. Evaluation at low temperatures will give erroneous results
- the sample should be assessed under low and high power magnification
- the assessment of motility is subjective, but the same observer can become very consistent
- samples should be assessed for the percentage of progressive motility. It is often easiest to categorize spermatozoal motility, using five groups:
  (a) category O: non-motile spermatozoa
  (b) category I: spermatozoa that are motile but not progressive
  (c) category II: spermatozoa that are motile but poorly progressive
  (d) category III: spermatozoa that are motile but moderately progressive
  (e) category IV: spermatozoa that are motile and rapidly progressive (swimming quickly in a forward direction)

NB: Using these criteria normal dogs have more than 70% spermatozoa with category IV motility.

- in the author's laboratory we have aimed to refine methods for the evaluation of sperm motility. Initial studies have examined sperm penetration, others investigated computer assessed swimming criteria and others the ability of sperm to exhibit normal motility characteristics and penetrate oocytes [5,6,7]
- this work is important as it has enabled translation of laboratory information into clinical practice
- motility will decrease with time when the slide is left on the microscope stage (probably due to the effect of light and cooling)

Sperm shape (morphology) (Figure 10.4)

This is the percentage of spermatozoa that conform to the shape accepted as normal for dog semen.

- morphology can be examined in wet preparations, but in general fixation and staining of the sperm is necessary
- a variety of stains have been described including Giemsa
- a simple method may be used that allows spermatozoal morphology and membrane integrity to be established at the same time; this is called 'vital staining'
- vital staining uses a simple stain, nigrosin–eosin, that is best refrigerated between use
- the eosin is taken up by cells that were dead at the time of staining (damaged membranes), and they therefore appear pink

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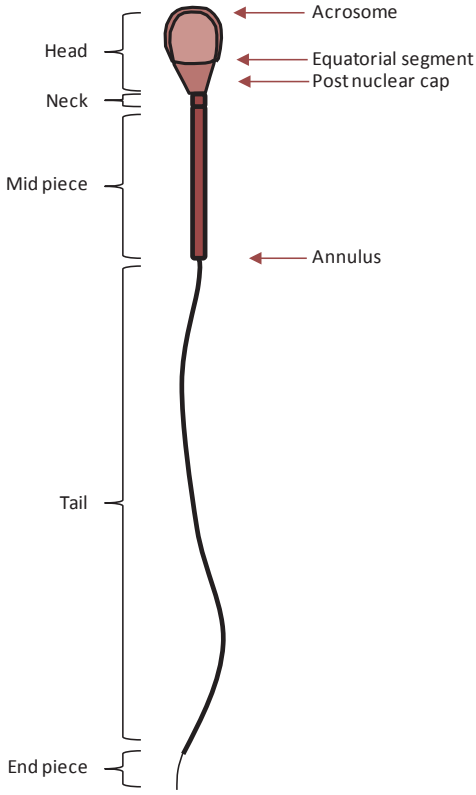


Figure 10.4 Schematic representation of the morphological appearance of a normal dog sperm.

- the nigrosin provides a background stain so that the spermatozoa are silhouetted against it and their shape can be seen; nigrosin has a purple–blue colour
- spermatozoa that appear white are classified as live; these sperm have intact membranes which prevent the eosin from penetrating into the sperm

NB: The head of the spermatozoon is bilaterally flattened like a table tennis bat.

Preparation of a sample for morphological examination with nigrosin–eosin

- place seven drops of nigrosin–eosin stain in a test tube and leave it to warm in the water bath for 2 minutes
- add one drop of semen (after mixing, as spermatozoa will have settled)
- immediately, with a fresh pipette, place one drop of stain–semen mixture onto a clean microscope slide

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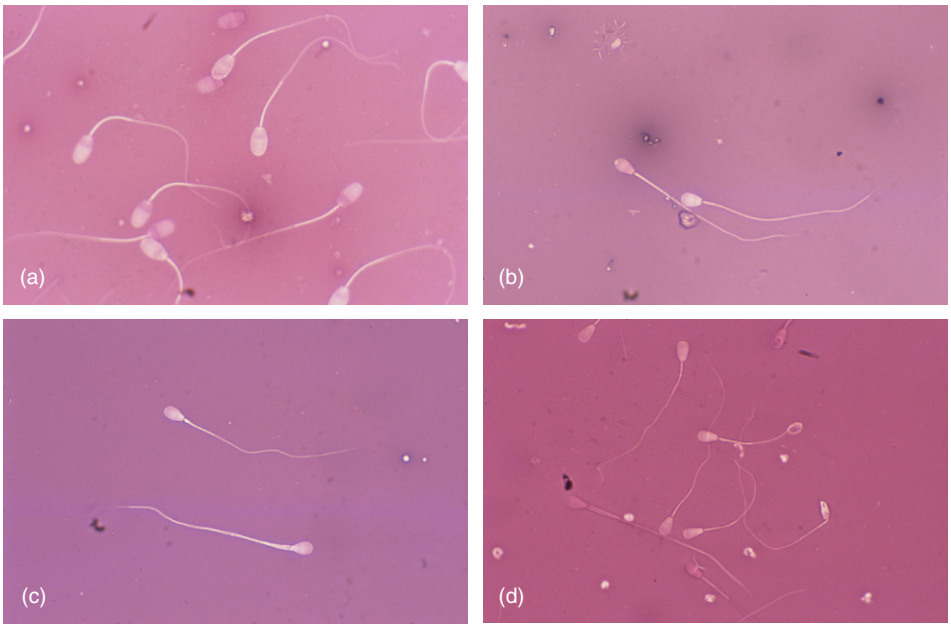


Figure 10.5 Microscopic examination of sperm after staining with nigrosin and eosin. Live sperm appear unstained (white) and dead sperm are stained pink by the eosin. Nigrosin provides the background colour. (a) The majority of sperm are live but have bent mid pieces. Detached heads are stained pink by eosin. (b) The dead (pink) sperm has a knobbed acrosome and the live (white) sperm is normal. (c) The top sperm is live but has a thickened mid piece, the lower sperm has a double mid piece. (d) There are multiple sperm defects in this sample.

- using a second slide at an angle of 45 degrees, draw a thin film of material across the first slide, as in preparing a smear for haematology
- allow the smear to dry (this is usually quite rapid)
- examine the slide under the ×100 objective lens using oil; if the spermatozoa are all overlying each other, another smear should be made using a larger volume of stain; if the spermatozoa are very sparse, another smear may be made using less stain
- the spermatozoa are seen in the smear silhouetted against the dark (nigrosin) background
- 100 spermatozoa are examined and classified as either alive and normal, dead or abnormal (Figure 10.5)

Classification of abnormal spermatozoa

- spermatozoal abnormalities can be classified into three categories:
  (a) primary abnormalities occur during spermatogenesis
  (b) secondary abnormalities occur during the epididymal phase of development
  (c) tertiary abnormalities occur during collection and processing the sample

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- classification of abnormalities may therefore be useful for elucidating their origin, and may provide information regarding the likely prognosis
- tables of primary, secondary and tertiary abnormalities are provided in standard textbooks
- the following are common abnormalities:
  (a) head abnormalities:
    – lifted (oedematous) acrosomes
    – knobbed acrosomes
    – crater defect
  (b) neck abnormalities:
    – bent neck
    – broken neck
  (c) mid piece abnormalities:
    – proximal cytoplasmic droplet
    – neck tag (disrupted mitochondria)
  (d) tail abnormalities:
    – distal cytoplasmic droplet (this is usually accepted as normal)
    – bent tail
    – coiled tail
  (e) spermatozoa which are dead at the time of staining are penetrated by the eosin (pink)

NB: Normal dogs will have more than 80% morphologically normal live spermatozoa within the ejaculate.

The author has noted that some commercially available nigrosin–eosin stains may actually cause bending of the tail and mid piece (presumably as a result of incorrect osmolarity) and therefore it is recommended that every new stain batch is carefully tested before being used for clinical diagnosis.

Other tests for sperm function

Several other tests are now used to assess spermatozoa, including:

The hypo-osmotic swelling test

- a test of functional integrity of the spermatozoal membrane
- sperm are exposed to hypo-osmotic conditions and water passes into the cytoplasm of sperm that have a functional membrane; these sperm develop bent tails
- sperm with non-functional membranes do not develop bending of the tail
- counting of the percentage of sperm with straight or bent tails after incubation in this medium correlates well to functional ability of sperm membranes (Figure 10.6)
- work in the author's laboratory developed this methodology for clinical practice in dogs and it has been widely implemented [8]

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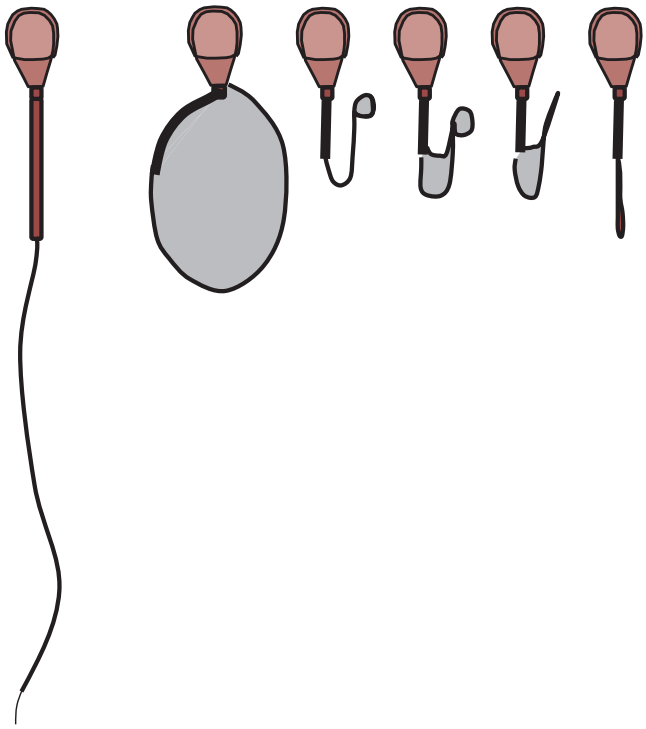


Figure 10.6 Morphological appearance of a non-swollen sperm and five sperm with different types of swelling when exposed to hypo-osmotic conditions. The swollen sperm have intact and functional membranes whereas the non-swollen sperm does not.

Sperm penetration assays

- a test of penetrating ability of the sperm (ability to acrosome-react and to change motility characteristics to a hyperactive form)
- pre-determined number of sperm are incubated with homologous oocytes (or oocytes from hamsters) and the numbers of oocytes penetrated and the number of penetrating sperm are counted
- in the author's laboratory we pioneered the use of homologous canine oocytes for sperm penetration assays and demonstrated the value and clinical applicability of this technique [6,9]
- in some cases simple attachment of sperm rather than penetrating ability is assessed

Sample storage

- always put some of the sample into buffered formal saline (roughly 1:1), wax the bottle cap to prevent evaporation and label the bottle clearly; this provides for the ability to retrospectively examine all samples in any cases of doubt

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Overall Interpretation (see also 17.3)

Polymorphonuclear neutrophils (PMNs, neutrophils) and macrophages

- these may come from the prostate gland but more commonly are contaminants from the penile wall and preputial lining (see 17.2)
- wiping the penis to remove this film of cells before collection is resented by most dogs
- spermatozoa can often be seen to be adherent to macrophages, but in general the motility of the sample is not affected by PMNs unless they are present in very large numbers

Erythrocytes

- these are commonly seen in the ejaculates of dogs over 5 years old
- they undoubtedly originate in the prostate gland, and are usually present in the absence of clinical disease
- even when present in large numbers, they do not appear to affect sperm motility at least for short periods of incubation (this is not the case in other species)

Motility

- is significantly affected by temperature and it is important that all evaluations are undertaken at a standard temperature otherwise variations in temperature between examinations may result in erroneous interpretation of motility
- motility can also be adversely affected by toxic substances that sperm are exposed to during collection (e.g. dusty collection equipment, water contamination of equipment, exposure to lubricating gel) [3]
- in many fertile dogs total motility is 90–95% with the percentage of normal motility being only slightly lower (i.e. the majority of sperm have normal forward progressive motility)
- less than 80% total motility and progressive motility of 60% or less may indicate a reduction in fertility

Morphology (Figure 10.5)

- the staining technique may affect morphology, e.g. some preparations of nigrosin–eosin cause bending of the sperm tail
- the sample should be stained as soon as possible after collection
- detailed estimation can only be made on samples examined at ×1000 (×100 lens and ×10 eyepiece) under oil
- less than 80% normal live spermatozoa may indicate a reduction in fertility and a clear relationship to poor fertility has been demonstrated when less than 60% sperm are morphologically normal at light microscopy

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- it is feasible that some abnormalities may not be detected by light microscopy
- the significance of specific sperm abnormalities is outlined below

Acrosome abnormalities

- the acrosome is essential for fusion of the sperm with the oocyte
- premature lifting or separation of the acrosome may indicate early capacitation (a maturation process which usually occurs when the sperm is in the female genital tract and must occur before the sperm can penetrate an ovum)
- knobbed acrosomes are usually only seen in small numbers in dog semen; in other species large numbers of knobbed acrosomes cause sterility
- the crater defect probably indicates that parts of the DNA (genetic material) in the head have not formed properly

Neck abnormalities

- bending at the neck or detachment of the head may be caused by rough handling of the sample; usually very few such sperm are present in any one sample
- proximal cytoplasmic droplets are unilateral smooth swellings at the proximal mid piece (neck); they are remnants of the cytoplasm of the sperm precursor, the spermatid
- spermatozoa in the head of the epididymis normally have proximal droplets; such spermatozoa are not immediately capable of fertilization; the presence of proximal droplets probably indicates accelerated passage of spermatozoa through the epididymis

Mid piece abnormalities

- in some dogs infertility is caused by disruption of the continuity of the neck and mid piece associated with abnormalities of the mitochondria; irregular swellings (tags) are seen at the neck and along the rest of the mid piece – these sperm usually have abnormal motility
- distal cytoplasmic droplets are located at the distal end of the mid piece and represent migration of the proximal droplet; they are considered common and do not appear to affect fertility

Tail abnormalities

- abnormal shaped tails do not allow progressive movement of the spermatozoon and may compromise both its journey to the uterine (Fallopian) tube, detachment of sperm from epithelium and its ability to penetrate the oocyte

Predicting fertility (see 17.3)

- the wide variety in semen abnormalities is interesting but tends to complicate the situation with respect to establishing a prognosis for fertility

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- some of the first published data concerning the large variation in semen quality from fertile dogs came from the author's clinic [10]
- surprising in this data were the poor-quality samples that originate from some fertile dogs

Comments about predicting fertility

- as long as the dog has not already ejaculated on the collection day, the time of his previous ejaculation has little effect on his semen quality; however, if a dog that appears ill-at-ease produces a sample of poor quality, a repeat examination is indicated since there may be incomplete ejaculation
- the dog may have received drugs which will affect semen quality (see Chapter 27)
- as a result of semen analysis, a dog cannot be said to be fertile; fertility includes the ability to mount and copulate normally and can only be proven if the dog produces offspring
- dogs with seminal values described above should be fertile, i.e. if mated once or twice to bitches close to ovulation time, and should produce litters of average size for that breed from 80% or more of the bitches that have been mated
- dogs with poor semen quality (low motility, low numbers, many abnormal sperm) will still be fertile, but more bitches will be expected to fail to become pregnant; repeated matings to the same bitch around her ovulation time may increase the likelihood of her conceiving
- dogs with no spermatozoa, or very small numbers of dead, abnormal spermatozoa, may:
  (a) have been treated with anabolic steroids or other androgens; their effect is usually reversible and the ejaculate should return to normal in 2 months
  (b) not have ejaculated properly, usually due to nervousness
  (c) have suffered a spermatogenic arrest (see 17.3); these dogs are initially fertile but suffer a rapid, asymptomatic testicular degeneration which results in permanent aspermia

10.6 MEASUREMENT OF ALKALINE PHOSPHATASE CONCENTRATIONS

- alkaline phosphatase is produced in high concentrations by the epididymis and therefore is present within the normal ejaculate at high concentrations (>5000 IU/l)
- measurement of alkaline phosphatase is useful when samples are found to be azoospermic
- semen samples can be sent to a commercial biochemistry laboratory for measurement of alkaline phosphatase
- azoospermic samples with low concentrations of alkaline phosphatase are either incomplete ejaculation, retrograde ejaculation or epididymal obstruction

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(rare in the dog). A repeated attempt of semen collection should be made followed by catheterization of the urinary bladder to look for sperm in urine which is found in cases of retrograde ejaculation
- azoospermic samples with high concentrations of alkaline phosphatase are confirmed cases of azoospermia

10.7 ULTRASONOGRAPHIC EXAMINATION OF THE MALE REPRODUCTIVE TRACT

Until relatively recently diagnostic ultrasound was overlooked for examination of the reproductive tract. There is no doubt, however, that this imaging modality should be part of a normal clinical examination of the male reproductive tract.

Equipment for examination of the dog

- linear transducers are most suited to scrotal imaging, but sector transducers are adequate
- sector transducers are most suited to transabdominal imaging
- a 7.5 or 10.0 MHz transducer is necessary to obtain suitable image definition but a 5.0 MHz transducer offers a compromise which gives a reasonable depth of penetration combined with adequate tissue resolution
- the principles of ultrasonography and ultrasound terminology are given in 5.8
- in some cases colour-flow ultrasonography or contrast-enhanced ultrasound are useful for examination of the prostate and testes [11,12]

Examination of the dog

- cleaning of the scrotum and application of a high viscosity gel is necessary to obtain good-quality images; clipping the scrotal hair should be avoided as this invariably traumatizes the scrotal skin and results in significant licking and self-trauma
- clipping the hair of the ventral abdomen is required for examination of the prostate gland
- in many patients imaging is most conveniently performed with the dog in the standing position

The normal testis and epididymis

- the author has described several imaging planes examination of the testes [13]:
  (a) transverse
  (b) sagittal
  (c) dorsal
  (d) pre-scrotal position (allows comparison of left and right testes)

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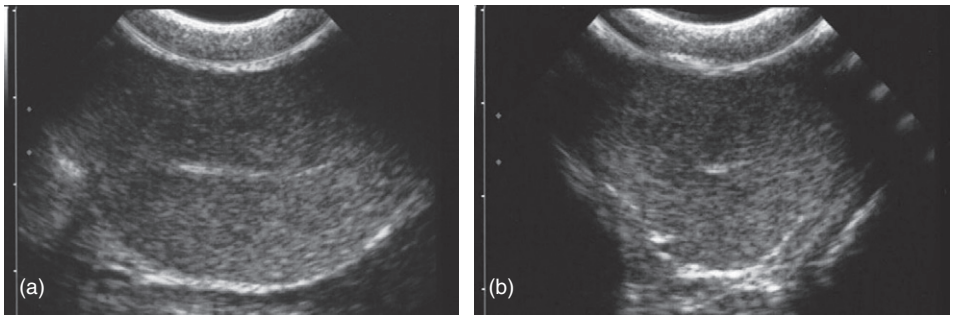


Figure 10.7 Ultrasound image: (a) the left testes in longitudinal section; the hypoechoic epididymides can be clearly seen. The central echogenic line within each testis is the mediastinum testis; (b) a single testis in transverse section; the mediastinum testis appears as a central echogenic spot. 7.5 MHz transducer, scale in centimetres.

- the testicular parenchyma appears relatively hypoechoic with regular diffuse echogenic stippling scattered evenly throughout the organ
- the stippling represents an extension of the fibrous mediastinum which is responsible for supporting the parenchymal tissue
- the mediastinum and stippled appearance may appear more pronounced when using higher frequency ultrasound transducers
- the mediastinum testis is located centrally within the tesis. In a sagittal plane this structure appears as an echogenic line approximately 2 mm wide extending from the cranial to the caudal pole, whilst in the transverse plane it appears as a central echogenic circular structure (Figure 10.7)
- acoustic shadowing is often noted distal to the mediastinum testis
- surrounding the testis the hyperechoic summed testicular and vaginal tunics are clearly defined
- the head and body of the epididymis are often difficult to identify
- the epididymal tail has a triangular-shaped appearance and is positioned on the caudal pole of the testis
- the epididymis appears hypoechoic with respect to testicular parenchyma

Relationship between testicular size and semen quality

- ultrasound can be used to accurately measure testicular size and to calculate testicular volume [13]
- the formula for calculation of testicular volume is:

$$\text{volume} = \frac{3}{4}\pi(\text{length} \times \text{width} \times \text{depth})$$

- importantly there is a good relationship between testicular volume and body size

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- in addition there is a general relationship between testicular size and semen quality, this is not always the case and some azoospermic dogs, and dogs with severe spermatozoal abnormalities may have a normal testicular volume

Relationship between testicular ultrasound appearance and semen quality

- whilst testes have a hypoechoic background and an obvious stippled pattern this appearance is homogenous in the normal dog
- testes which appear more heterogenous are often associated with poor semen quality (Figure 10.8)
- testes which appear overall to be more hypoechoic than normal are often associated with poor semen quality

Relationship between ultrasound appearance and testicular pathology

- we have described a range of different testicular and scrotal pathologies that can be detected using real-time ultrasound examination [13,14]

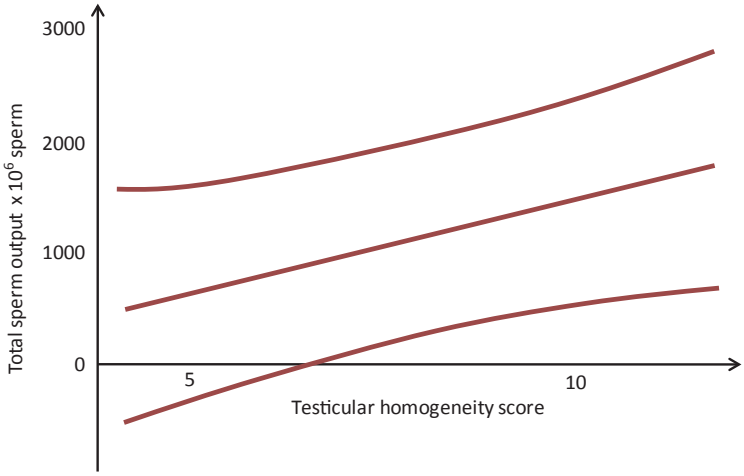


Figure 10.8 Mean and confidence limits for testicular homogeneity score vs total sperm output; testes which are more homogenous are associated with a higher total sperm output.

The normal prostate gland

- examination of the prostate gland is facilitated by the presence of fluid within the bladder
- this can be achieved by preventing urination, or the infusion of sterile isotonic saline via a catheter

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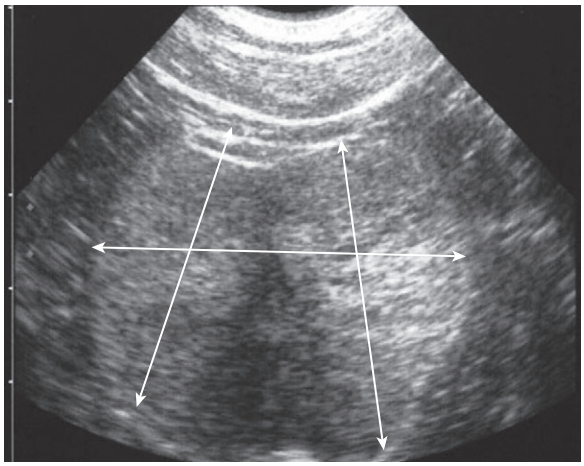


Figure 10.9 Transverse section ultrasound image of normal dog prostate demonstrating total width and height of the left and right lobes.

- imaging is achieved by placing the ultrasound transducer on the caudo-ventral abdomen adjacent to the prepuce
- imaging may be difficult if the gland lies entirely within the pelvis as may be the case in a young dog where where the gland is small; however, the transducer may be placed into the dog's rectum and directed ventrally to allow adequate imaging of the caudal prostate
- the normal prostate gland is well circumscribed, although frequently the prostatic capsule is difficult to identify because its specular echo is sensitive to the direction of the ultrasound
- the gland usually has a symmetrical bi-lobed outline
- there is a midline furrow dorsal to the prostatic urethra
- the prostatic parenchyma is moderately echogenic, and has coarse stippling present evenly throughout the gland (Figure 10.9)
- at the hilar region the prostate has linear echogenic streaks associated with peri-urethral tissue
- the prostatic urethra is often visible only when the ultrasound strikes it at 90 degrees
- in dogs that are sedated or anaesthetized the urethra may be urine filled and therefore appears as an anechoic line in longitudinal section
- there is a weak correlation between body size and volume of the prostate gland
- in the castrated male the prostate is small and hypoechoic, the smallest size being found in dogs castrated before puberty

Relationship between ultrasound appearance and semen quality

- prostatic heterogeneity does not correlate with semen quality
- prostatic echogenicity does not correlate with semen quality

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- a large prostatic volume is associated with a larger volume of prostatic fluid at the time of ejaculation

The use of colour-flow and contrast-enhanced ultrasound

- it can be difficult to determine the difference between benign and malignant lesions of the testes and prostate
- there are however difference in vascularization which may be demonstrated using colour-flow and contrast-enhanced ultrasonography; we have published baseline normal values and shown how these are perturbed in clinical cases of prostate pathology [11,12]
- generally perfusion parameters are higher for neoplastic lesions

10.8 MEASUREMENT OF PLASMA HORMONES

Measurement of basal plasma (serum) hormone concentrations has limited application in the infertile dog.

Plasma (serum) testosterone concentration

- testosterone concentrations vary widely throughout the day
- single samples are often not diagnostic
- basal testosterone is only useful for the demonstration of functional testicular tissue
  (a) entire dogs have testosterone concentrations above 4.0 ng/ml (14.0 nmol/l)
  (b) castrated dogs have testosterone concentrations below 2.0 ng/ml (7.0 nmol/l)
  (c) dogs with impaired spermatogenesis have values similar to entire dogs
  (d) bilaterally cryptorchid dogs have values similar to entire dogs

NB: Single samples from entire dogs may be taken at the 'trough' of daily production and therefore appear basal.

Plasma (serum) luteinizing hormone (LH) concentration

- LH is released episodically approximately every 90 minutes
- LH concentrations can fluctuate by up to four times basal secretion during the day
- increased LH concentrations may develop in cases of impaired spermatogenesis
  (a) normal dogs have LH concentrations below 30 ng/ml
  (b) castrated dogs have LH concentrations above 70 ng/ml

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(c) dogs with impaired spermatogenesis have LH concentrations above 70 ng/ml
(d) bilaterally cryptorchid dogs have values similar to entire dogs

NB: Preferably, mean values should be calculated after collecting serial samples over time.

Plasma (serum) follicle-stimulating hormone (FSH) concentration

- FSH release fluctuates during the day but to a lesser degree than LH
- increased FSH concentrations may develop in cases of impaired spermatogenesis
  (a) normal dogs have FSH concentrations below 70 ng/ml
  (b) castrated dogs have FSH concentrations above 700 ng/ml
  (c) dogs with impaired spermatogenesis have FSH concentrations above 100 ng/ml
  (d) bilaterally cryptorchid dogs have values similar to entire dogs

NB: Preferably, mean values should be calculated after collecting serial samples over time.

10.9 OBSERVATION OF MATING BEHAVIOUR

- it is always important to observe mating attempts in dogs that are said to be infertile
- common difficulties at otherwise normal mating are described in 9.4

10.10 BACTERIAL EXAMINATION OF THE MALE REPRODUCTIVE TRACT

- all male dogs have a resident commensal bacterial flora
- this may be associated with a persistent or recurrent mucopurulent discharge
- the bacteria isolated may change daily
- many species of bacteria are present, including haemolytic streptococci, staphylococci, other streptococci, Escherichia coli, and many others
- there is usually a mixed flora with two to five species present
- the incidence of anaerobic bacteria is not well documented

Interpretation of bacteriology of the prepuce

- these swabs should be expected to contain bacteria
- there is no evidence that this is a sign of clinical disease
- there is no evidence in a change in the flora following coitus

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- withholding mating because of the isolation of a commensal bacterium (including β-haemolytic streptococci) seems unnecessary
- the only known bacterial venereal pathogen is Brucella canis
- dogs in countries where Brucella is present should be routinely screened for this organism
- otherwise routine bacteriological screening of the clinically normal dog is pointless

10.11 TESTICULAR BIOPSY

- biopsy allows the evaluation of spermatogenesis, and the detection of inflammatory and neoplastic cells
- biopsy is, however, very invasive and can itself cause severe testicular changes
- excisional wedge biopsy with closure of the tunica albuginea is the least traumatic method
- tissue should be fixed in Bouin's fixative and examined by an experienced histopathologist

10.12 TESTICULAR FINE-NEEDLE ASPIRATION

- requires sedation preferably with a sedative/analgesic compound
- aspiration of the testicular parenchyma using a 20G needle attached to a 10 ml syringe usually gives an adequate sample
- material should be immediately fixed and stained for examination by an experienced histopathologist
- a variety of cell types including spermatocytes, spermatids and spermatozoa may be identified; Sertoli cells are easily recognized, but in normal dogs limited numbers of Leydig cells and spermatogonia are found
- subjective scoring of the percentages of each cell type may be useful when investigating infertile dogs

10.13 PROSTATIC BIOPSY

- true cut biopsies can be obtained in the anaesthetized dog using a transabdominal or pararectal approach
- wedge biopsy at laparotomy is the preferred method because the procedure also allows inspection of the prostate gland; this ensures that a representative sample is collected
- ultrasound-guided fine-needle aspiration may also be useful in allowing the collection of tissue; one particular advantage is the rapid collection of material for bacteriological examination in cases of prostatic abscessation
- collection of the third fraction of the ejaculate should not be overlooked as a technique allowing the bacteriological and cytological evaluation of the prostate

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10.14 PROSTATIC MASSAGE/URETHRAL FLUSHING

- prostatic fluid for bacteriological and cytological evaluation can be obtained by massage of the prostate transrectally whilst simultaneously flushing the prostatic urethra with 5–10 ml of physiological saline
- examination of pre- and post-massage aspirates of the urethra ensures that any cells identified originate from the prostate

10.15 ENDOCRINOLOGICAL STIMULATION TESTS (Figure 10.10)

- measurement of basal hormone concentrations is not always diagnostic
- hormone stimulation tests may be useful to confirm the presence of a functional pituitary–testicular interaction (the gonadotrophin-releasing hormone (GnRH) stimulation test)
- hormone stimulation tests may be useful to confirm the presence of functional testicular tissue (the human chorionic gonadotrophin (hCG) stimulation test)

The gonadotrophin-releasing hormone (GnRH) stimulation test

- a plasma (serum) sample is collected and assayed for LH and testosterone concentration
- a GnRH analogue is injected intravenously (0.01 mg/kg buserelin) and a second sample is assayed for LH and testosterone concentration 60 minutes later
- a significant increase in LH concentration indicates the presence of a functional pituitary gland
- a significant increase in testosterone concentration indicates the presence of a functional pituitary gland and testicle
- the test has not been widely evaluated and it is more common to perform an hCG stimulation test

The human chorionic gonadotrophin (hCG) stimulation test

- in the author's laboratory we have determined the response of plasma testosterone to hCG in normal dogs and dogs with no testes; this now forms the basis of a diagnostic test [15]
- hCG is used rather than LH because the latter is not commercially available
- hCG is principally LH-like in activity
- a plasma (serum) sample is collected and assayed for testosterone concentration
- hCG is injected intravenously (50 IU/kg) and a second sample is assayed for testosterone concentration 60 minutes later
- a significant increase in testosterone concentration indicates the presence of functional testicular tissue (the test may be used to confirm the presence or

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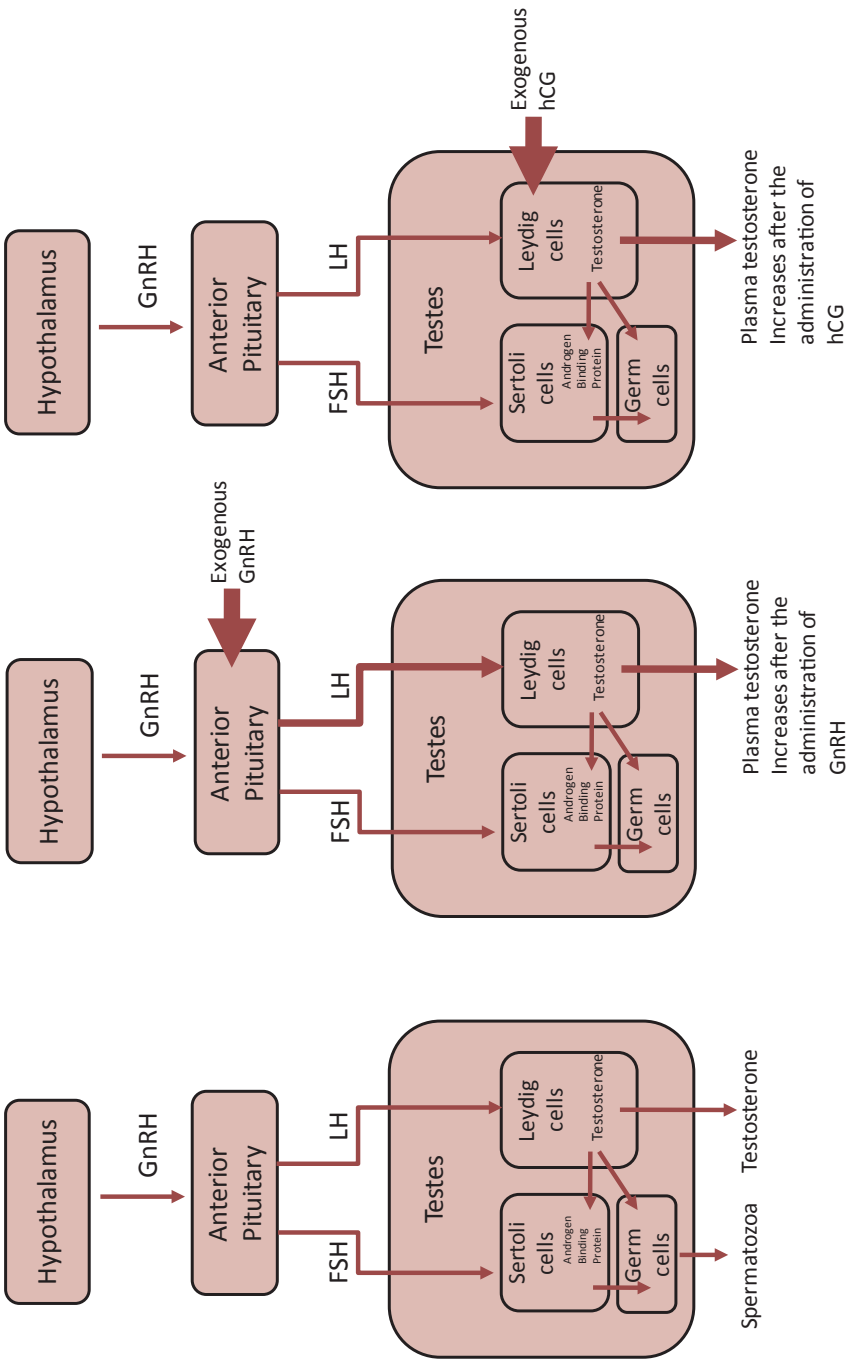


Figure 10.10 Injection of GnRH and hCG causes increased secretion of testosterone in the normal dog.

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absence of testicular tissue in dogs with an unknown reproductive history, e.g. apparent bilateral cryptorchidism)

10.16 KARYOTYPING

- this is establishing the number and appearance of chromosomes
- may be useful in certain cases of infertility especially in males with ambiguous external genitalia
- a limited number of institutions offer this service
- requires collection of blood into EDTA anticoagulant and rapid transport to the laboratory
- chromosomes are examined from metaphase spreads following culture
- the most common abnormalities seen in the dog are:
  (a) XX sex reversal seen in the American cocker spaniel breed; here the external genitalia are small and malformed often with hypospadius (see 17.2)
  (b) Male pseudo-hermaphrodite where the genitalia are male including bilateral testes but there are internal female tubular genitalia

REFERENCES

1 England, G.C.W. (1995) Evaluation of seminal characteristics in the dog. *Journal of Animal Breeding* **1**, 38–45.
2 England, G.C.W. (1999) Semen quality in dogs and the influence of a short-interval second ejaculation. *Theriogenology* **52**, 981–986.
3 England, G.C.W. and Allen, W.E. (1992) Factors affecting the viability of canine spermatozoa I. Potential influences during processing for artificial insemination. *Theriogenology* **37**, 363–371.
4 England, G.C.W. and Allen, W.E. (1992) Factors affecting the viability of canine spermatozoa II. Effects of seminal plasma and blood. *Theriogenology* **37**, 373–381.
5 England, G.C.W. and Allen, W.E. (1990) Evaluation of cellulose acetate/nitrate filters for measuring the motility of dog spermatozoa. *Journal of Reproduction and Fertility* **88**, 369–374.
6 Hewitt, D.A., Fletcher, S. and England, G.C.W. (2001) Test of canine sperm function in vitro using primary homologous oocytes and confocal microscopy. *Journal of Reproduction and Fertility* **57**(Supplement), 127–136.
7 Smith, S.C. and England, G.C.W. (2001) Effect of technical settings and semen handling upon motility characteristics of dog sperm measured using computer-aided sperm analysis. *Journal of Reproduction and Fertility* **57**(Supplement), 151–159.
8 England, G.C.W. and Plummer, J.M. (1993) Hypo-osmotic swelling of dog spermatozoa. *Journal of Reproduction and Fertility* **47**(Supplement), 261–270.
9 Hewitt, D.A. and England, G.C.W. (1997) The canine oocyte penetration assay: its use as an indicator of dog spermatozoal performance in vitro. *Animal Reproduction Science* **50**, 123–139.
10 England, G.C.W. and Allen, W.E. (1989) Fertility and semen parameters in dogs. *Veterinary Record* **125**, 399.

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11 Russo, M., Vignoli, M., Catone, G., Rossi, F., Attanasi, G. and England, G.C.W. (2009) Prostatic perfusion in the dog using contrast-enhanced doppler ultrasound. *Reproduction in Domestic Animals* **44**, 334–335.
12 Vignoli, M., Russo, M., Catone, G., Rossi, F., Attanasi, G., Terragni, R., Saunders, J.H. and England, G.C.W. (2011) Assessment of vascular perfusion kinetics using contrast-enhanced ultrasound for the diagnosis of prostatic disease in dogs. *Reproduction in Domestic Animals* **46**, 209–213.
13 England, G.C.W. (1991) The relationship between ultrasonographic appearance, testicular size, spermatozoal output and testicular lesions in the dog. *Journal of Small Animal Practice* **32**, 306–311.
14 Ahmad, N., England, G.C.W. and Noakes, D.E.N. (2000) Sequential ultrasonography of spontaneously-occurring lesions of the genital system of rams, and their influence on semen quality. *Veterinary Record* **146**, 10–15.
15 England, G.C.W., Allen, W.E. and Porter, D.J. (1989) The evaluation of the testosterone response to human chorionic gonadotrophin, and the identification of a presumed anorchid dog. *Journal of Small Animal Practice* **30**, 441–443.

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11 Fertilization and the Optimal Breeding Time

It is imperative to understand the normal physiology of the bitch, especially that associated with normal fertilization, since there are considerable differences to the other domestic species. Misunderstanding of the normal physiology on behalf of the breeder is undoubtedly the most common cause of infertility in dogs.

11.1 MATING TIME AND FERTILIZATION TIME

- whilst bitches may appear to be very similar in the manifestation of pro-oestrus and oestrus, bitches differ significantly in when ovulation occurs within the cycle
- in the author's laboratory we documented for the first time the fact that ovulation could normally be as early as day 5 after the onset of pro-oestrus, or as late as day 30 after the onset of pro-oestrus [1]
- these data are important because they have changed clinical practice in the way we manage breeding time in bitches
- within the same bitch there are also differences in the time of ovulation from one cycle to the next
- determining when to breed by counting the number of days from the onset of pro-oestrus is inaccurate since the time of ovulation is variable in relation to the onset of pro-oestrus (see Figure 3.2)
- this information has been known for 20 years and yet many breeders impose standard mating regimes (usually mating a defined number of days after the onset of vulval bleeding; say day 10 and day 12)
- this results in many bitches being mated at an inappropriate time, and this constitutes the commonest cause of apparent infertility
- fortunately, both eggs and spermatozoa have prolonged survival in the female reproductive tract (this results in the differences between the 'apparent' and the 'actual' pregnancy length; see 12.1) and this may overcome to some degree the error on behalf of the breeder

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11.2 REPRODUCTIVE PHYSIOLOGY

- whilst the time of ovulation is variable in relation to pro-oestrus and oestrus the hormonal events surrounding ovulation and the physiology of the oocyte are tightly regulated such that these occur in a well defined time sequence
- oestrogen produced by the developing follicles reaches stable concentrations for 2 days before starting to decline
- approximately 2 days after oestrogen concentrations start to decline there is a short-lived surge in plasma gonadotrophins (principally LH, so frequently this is termed the LH surge)
- coincidental with the LH surge there is demonstrable increase in plasma progesterone concentrations (progesterone is produced by luteinization of the inner wall of the follicle prior to ovulation)
- the process of ovulation commences 2 days after the LH surge
- the oocyte is ovulated in an immature state, and unlike other species cannot be fertilized immediately
- fertilization can only occur following maturation of the primary oocyte, extrusion of the polar body and completion of the first meiotic division
- occytes become fertilizable 2 days after ovulation
- oocytes remain viable within the reproductive tract for a further 4–5 days before degenerating
- it is important to remember that sperm are also able to survive within the female reproductive tract for many days
- in the author's clinic we have undertaken pivotal studies investigating the interaction between sperm and the female reproductive tract [2,3]
- it is clear that sperm form a reservoir by attachment to epithelium at the tip of the uterine horns/distal uterine tube; from here sperm wait for specific signals that cause them to detach and search for an oocyte [4]
- understanding this 'triggering' of sperm maturation adds to biological knowledge and also offers potential targets to improve fertility and also possibly for contraception

Two distinct periods of time termed the 'fertile period' and the 'fertilization period' can be defined (Figure 11.1) [5].

The 'fertilization period'

- this is the time when oocytes can be fertilized
- the fertilization period commences 2 days after ovulation (i.e. 4 days after the pre-ovulatory surge of LH and 4 days after progesterone is first detected in circulation)
- the fertilization period ends when oocytes start to degenerate and the cervix become non-patent; this is generally approximately 5 days after ovulation (i.e. 7 days after the pre-ovulatory surge of LH)

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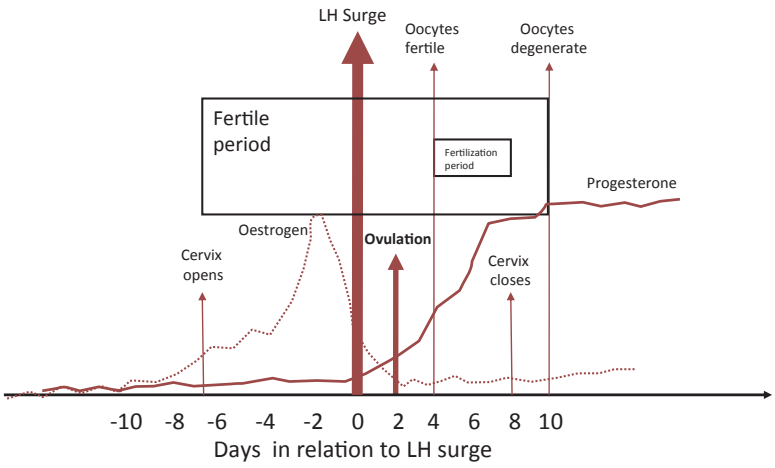


Figure 11.1 Schematic representation of the fertile and fertilization periods with reference to hormonal and physical changes of the female reproductive tract.

- the fertilization period therefore extends between 2 and 5 days after ovulation (i.e. between 4 and 7 days after the LH surge)

The 'fertile period'

- this is the time during which a mating could result in a conception
- the fertile period can start before oocytes become fertile because it is possible for sperm to be present within the female reproductive tract 'waiting' for the eggs to become fertilizable
- since sperm from an average fertile dog can survive for 7 days or more, the fertile period often commences 5 days before ovulation (sperm 'wait' for 5 days until eggs are released and a further 2 days until they become fertilizable)
- for some stud dogs sperm are able to survive for longer periods of time, whilst in others, or when preserved semen has been used, the sperm are able to survive only for a short time
- the fertile period terminates when oocytes degenerate and so it ends at the same time as the end of the fertilization period
- for an average fertile stud mating a bitch the fertile period therefore starts 5 days before ovulation and lasts until 5 days after ovulation (i.e. 3 days before the LH surge until 7 days after the LH surge)
- for many dogs mating during the broad fertile period will result in a pregnancy, however when semen quality is poor mating must occur during the fertilization period
- work in our laboratory was the first to accurately quantify fertilization rates in dogs [6]; these data show high rates in normal dogs and provide a baseline to measure abnormal cycles against

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11.3 ASSESSING THE OPTIMAL TIME FOR MATING [7]

- the optimal mating time is best considered to be during, or immediately preceding the fertilization period
- in clinical studies the period of peak fertility for natural matings has been confirmed to be from 1 day before, until 6 days after, the LH surge, which fits what is expected from understanding of the physiology
- it is very important to remember, however, that the time of ovulation cannot be reliably predicted by counting the number of days from the onset of pro-oestrus and neither can it be predicted by confirmation of a change to oestrous behaviour (i.e. by teasing with a stud dog)
- accurate determination of the optimal time to mate can therefore only be assessed by documenting the LH surge, or by methods that reliably indicate the fertile or preferably the fertilization period (including measurement of plasma progesterone, or assessment of changes of the reproductive tract which are caused by fluctuations in hormonal concentrations)
- when the use of poor-quality semen is contemplated (for example the use of preserved semen), insemination should be performed only during the fertilization period, to ensure success

Clinical assessments

Counting the number of days

- many dogs breeders rely upon counting the number of days from the onset of pro-oestrus
- many dog breeders believe that bitches always ovulate a defined number of days from the onset of this pro-oestrus; this is not true
- while the 'average bitch' may ovulate 12 days after the onset of pro-oestrus
  (a) some bitches ovulate as early as day 5 after the onset of pro-oestrus
  (b) some bitches ovulate as late as day 30 after the onset of pro-oestrus (Figure 3.2)
- mating on the 12th and 14th days, which is common breeding practice, may therefore fail to result in conception

Oestrous behaviour

- the classical definition of oestrus is a change in female behaviour from attractiveness of the male but refusal to allow mating to acceptance of mating
- the onset of oestrus can easily be demonstrated in the bitch by teasing with a male to look for a change in this behaviour
- whilst initial studies observing laboratory beagles suggested that the onset of standing oestrus occurred at the same time as the LH surge this is definitely not the case in many bitches, especially those that live in human households

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- if these data were always reliable it would be relatively easy to determine when to breed since the optimal time would commence 4 days later (2 days after the LH surge would be ovulation, and 2 days later would be when oocytes became fertilizable)
- we now know however that in many bitches the behavioural events correlate poorly with the underlying hormonal events such that assessing teasing behaviour alone does not result in a high pregnancy rate

Vulval softening

- during pro-oestrus there are elevated concentrations of oestrogen which result in the vulva and perineal tissues becoming enlarged, oedematous and somewhat turgid
- there is a dramatic decline in oestrogen concentrations which reach low values at the time of the LH surge (Figure 11.2)
- the removal of the effects of oestrogen result in distinct vulval softening which is pronounced at the time of the LH surge
- the first day that vulval softening is detected can therefore be used as an indicator of the day of the LH surge
- mating should therefore commence 4 days after the onset of vulval softening (2 days after the LH surge would be ovulation, and 2 days later would be when oocytes became fertilizable)
- when clinical assessments alone are available, the combination of the onset of standing oestrus and the timing of vulval softening may be useful because each event occurs, on average, 4 days before the beginning of the fertilization period but other methods are substantially more reliable



Figure 11.2 Dramatic softening of the vulva noted at 1 day after the LH surge.

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Endocrine assessment

Measurement of plasma LH (see 5.9)

- we have documented that measurement of the plasma concentration of LH is a reliable and accurate method for determining the optimum time to mate [8]
- LH can also be measured in urine, but concentrations are low and this assay is unreliable
- there is, however, no readily available commercial assay for canine LH

Measurement of plasma progesterone

- in the bitch, plasma progesterone concentration begins to increase from baseline approximately 2 days before ovulation (the result of pre-ovulatory luteinization) at approximately the same time as the LH surge
- progesterone concentrations are substantially elevated and easily detectable on the day of ovulation (values are often between 3.0 and 5.0 ng/ml depending upon the assay)
- in our laboratory we have documented the rise in progesterone and demonstrated that its measurement can be clinically useful for determining the optimal time to breed and for improving pregnancy rates and litter size [9]
- serial monitoring of plasma progesterone therefore allows:
  (a) anticipation of ovulation
  (b) confirmation of ovulation
  (c) detection of the fertilization period
- the initial rise in progesterone is gradual; therefore it is only necessary to collect blood samples every second or third day
- mating or insemination should be planned between 4 and 6 days after the plasma progesterone concentration exceeds 2.0 ng/ml (6.5 nmol/l) (the value at the time of the LH surge), or should preferably commence 1 day after values exceed 8–10.0 ng/ml (25.0–32.0 nmol/l) (the beginning of the fertilization period) (Figure 11.3)
- progesterone may be measured by radioimmunoassay or an enzyme-linked immunosorbent assay (ELISA) test kit (see 5.9)
- various test kits are available commercially and can be run within the practice laboratory. These kits can be semi-quantitative or qualitative and either offers some advantages and disadvantages [10]

NB: The author has demonstrated the possibility of running ELISA tests for progesterone using whole blood and vaginal fluid which removes the need for centrifugation [11,12].

Vaginal cytology (see 5.5)

- the change in concentrations of oestrogen and progesterone during pro-oestrus, oestrus and the early luteal phase cause substantial effects upon the reproduc-

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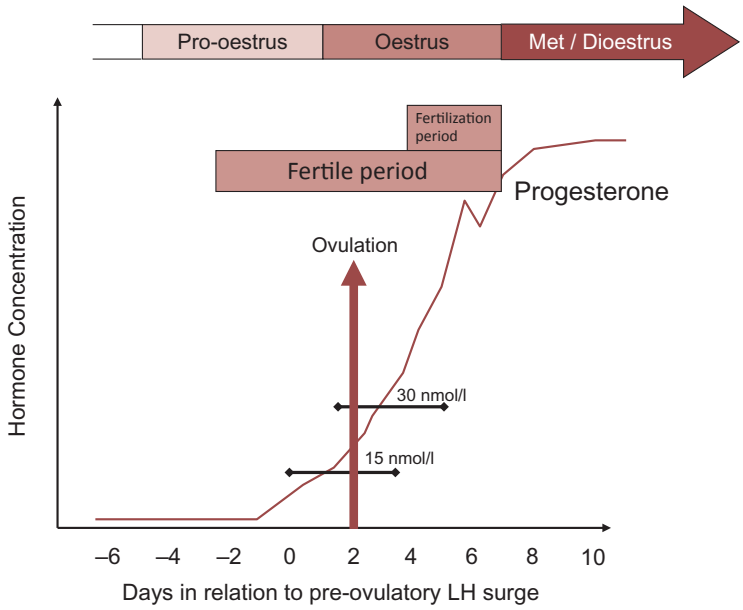


Figure 11.3 Schematic representation of the changes in progesterone profile in relation to the fertile and fertilization periods of the bitch.

tive tract which are especially manifest in the type of epithelium within the vagina

- collection, staining and microscopic examination of exfoliated vaginal cells is a simple method for monitoring the stage of the oestrous cycle
- the relative proportions of different types of epithelial cell can be used as markers of the endocrine environment
- the fertile (not fertilization) period can be predicted by calculating the percentage of epithelial cells that appear anuclear when using a modified Wright–Giemsa stain
- mating should be attempted throughout the period when:
  (a) more than 80% of epithelial cells are anuclear (the fertile period) (Figure 11.4)
  (b) polymorphonuclear neutrophils (PMNs) are absent from the smear
- at the end of the fertilization period, plasma progesterone concentrations remain high and there is sloughing of much of the vaginal epithelium
- the termination of the fertile period can be detected when:
  (a) the percentage of large irregularly shaped anuclear cells decreases
  (b) PMNs reappear in large numbers
  (c) the vaginal smear is dominated by small epithelial cells, cellular debris and bacteria

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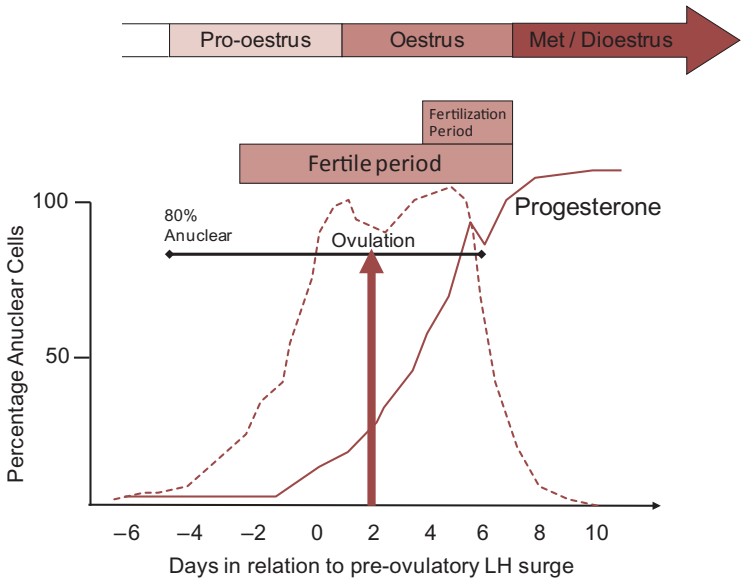


Figure 11.4 Schematic representation of the changes in the percentage anuclear vaginal epithelial cells in relation to the fertile and fertilization periods of the bitch.

- whilst vaginal cytology is a useful technique it should be remembered that:
  (a) PMNs may be found throughout the fertile period in some bitches
  (b) in some bitches peak values of only 60% anuclear cells are reached

Crystallization of cervico-vaginal mucus

- work in our laboratory has shown that mucus is present within the vaginal smear (if collected by aspiration), and can be examined by microscopic inspection of unstained smears [13]
- the mucus originates from glandular tissue within the cervix and cranial vagina and changes in its arborization pattern as the bitch moves from pro-oestrus to oestrus; in oestrus there are large parallel branches of the crystallization pattern and greater areas of the slide are covered with mucus crystals (see 5.5)
- our studies demonstrate that this technique can be clinically useful [14]

Vaginal endoscopy (see also 5.7)

- vaginoscopic assessment is based upon observation of:
  (a) mucosal fold contours and profiles
  (b) the colour of the mucosa
  (c) the colour of any fluid present

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- bitches progress through various stages of mucosal proliferation, oedema, swelling, shrinkage, angulation and decline (denoted I, O, S1, S2, A1, A2, A3, D1, D2, D3, D4, I) (see 5.7)
- this proliferation and regression is repeatable between bitches and we have shown that it can be used to accurately stage the oestrous cycle [8,15]
- all bitches reach the stage of marked furrowing, wrinkling and indentation (denoted S2) at 2 days before ovulation, and shrinkage with angulation such that the peaks are sharp-tipped and irregular in appearance (A2) at 4 days after ovulation (Figure 11.5)

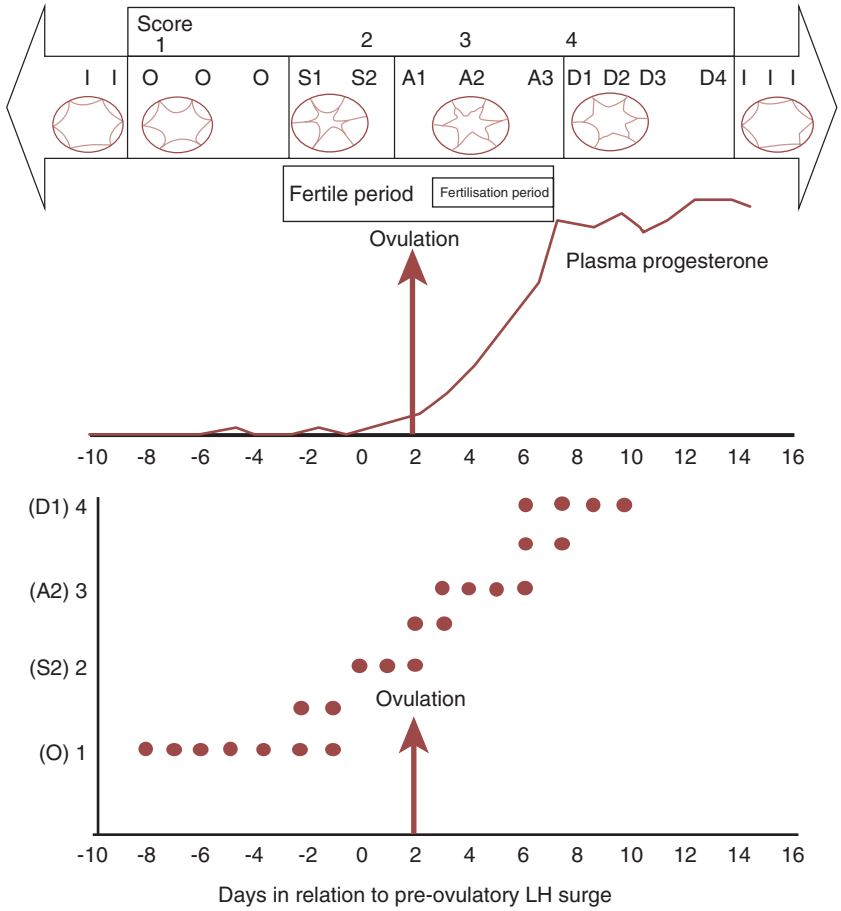


Figure 11.5 Changes in vaginoscopic appearance in relation to the fertile and fertilization periods (top panel) and scored parameters for three bitches on consecutive days of the cycle (bottom panel) (note on many days the vaginscopic score was identical and therefore the point overlap). All bitches reached mucosal scare S2 on 2 days before ovulation, A2 on 4 days after ovulation and D1 on 9 days after ovulation.

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- breeding should be planned on at the appearance of A1 stage, with breeding being ideal on the first day of stage A2 which is in the middle of the fertilization period
- the method is simple to perform, inexpensive and reliable for determining the optimal time to breed

Additional examination of cervico-vaginal secretion

- the electrical resistance of the vaginal secretions decreases during late oestrus, but measurement of electrical resistance has not been proven to be a reliable indicator of the optimal time to breed, indeed in individual bitches electrical resistance can vary haphazardly without any relation to the underlying endocrine environment
- some workers claim that the concentration of glucose within the vaginal discharge can be used to predict the optimal mating time, but the technique has failed to stand up to scientific investigation
- vaginal pH and glucose concentration also do not reliably correlate with stage of the oestrous cycle in a way to be clinically useful
- crystallization of mucus collected from the anterior vagina may be useful in combination with vaginal cytology for determining the optimal mating time (see Crystallization of cervico-vaginal mucus in this section, and 5.5)

REFERENCES

1 England, G.C.W., Allen, W.E. and Blythe, S.A. (1989) Variability of the time of calculated luteinising hormone release in 218 canine pregnancies. *Veterinary Record* **125**, 624–625.
2 England, G.C.W. and Pacey, A.A. (1999) Transportation and interaction of dog spermatozoa within the reproductive tract of the bitch. *Centre for Reproductive Biology* **3**, 57–84.
3 Pacey, A.A., Freeman, S.L. and England, G.C.W. (2000) Contact of dog spermatozoa with homologous uterine tube epithelium prolongs flagellar activity in relation to the stage of the estrus cycle. *Theriogenology* **54**, 109–118.
4 Brewis, I.A., Morton, I.E., Moore, H.D.M. and England, G.C.W. (2001) Solubilised zona pellucida proteins and progesterone induce calcium influx and the acrosome reaction in capacitated dog spermatozoa. *Molecular Reproduction and Development* **60**, 491–497.
5 England, G.C.W., Burgess, C.M., Freeman, S.L., Smith, S.C. and Pacey, A.A. (2006) Relationship between the fertile period and sperm transport in the bitch. *Theriogenology* **66**, 1410–1418.
6 England, G.C.W., Russo, M. and Freeman, S.L. (2009) Follicular dynamics, ovulation and conception rates in bitches. *Reproduction in Domestic Animals* **44**, 53–58.
7 Hewitt, D.A. and England, G.C.W. (2000) Assessment of optimal mating time in the bitch. *Journal of Postgraduate Clinical Study In Practice* **22**, 24–33.

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8 Jeffcoate, I.A. and England, G.C.W. (1997) Urinary LH, plasma LH and progesterone and their clinical correlates in the periovulatory period of domestic bitches. *Journal of Reproduction and Fertility* **51**(Supplement), 267–275.
9 England, G.C.W., Allen, W.E. and Porter, D.J. (1989) A comparison of radioimmunoassay with quantitative and qualitative enzyme–linked immunoassay for plasma progestogen determination in bitches. *Veterinary Record* **125**, 107–108.
10 Moxon, R., Copley, D. and England, G.C.W. (2010) Technical and financial evaluation of assays for progesterone in canine practice in the UK. *Veterinary Record* **167**, 528–531.
11 England, G.C.W. (1991) ELISA determination of whole blood and plasma progestogen concentration in bitches. *Veterinary Record* **129**, 221–222.
12 England, G.C.W. and Anderton, D.J. (1992) Determination of progestogen concentrations in the vaginal fluid of bitches in oestrus. *Veterinary Record* **130**, 143–144.
13 England, G.C.W. and Allen, W.E. (1989) Crystallization patterns in anterior vaginal fluid from bitches in oestrus. *Journal of Reproduction and Fertility* **86**, 335–339.
14 England, G.C.W. (1992) Vaginal cytology and cervico vaginal mucus arborisation in the breeding management of bitches. *Journal of Small Animal Practice* **33**, 577–582.
15 Moxon, R., Batty, H., Irons, G. and England, G.C.W. (2012) Periovulatory changes in the endoscopic appearance of the reproductive tract and teasing behavior in the bitch. *Theriogenology* (in press).

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12 Pregnancy and Lactation

12.1 PREGNANCY LENGTH

The 'apparent' pregnancy length

- this is usually taken to be the interval between the first mating and parturition
- fertile matings have been recorded between 9 days before and 5 days after ovulation
- the apparent length of pregnancy can therefore vary between 58 days and 72 days

The 'actual' pregnancy length

- the interval between ovulation and the day of parturition is consistently 62–64 days; this is termed the actual pregnancy length
- the day of onset of parturition can therefore be predicted by detection of events that occur during oestrus (for example parturition occurs 64–66 days after the pre-ovulatory LH surge, 62–64 days after ovulation, 61–63 days after progesterone concentrations reach high values)
- on average parturition occurs 57 days after the onset of cytological metoestrus (see 3.5 and 5.5) but this relationship is not as reliable as measurement of LH or progesterone
- progesterone concentrations are elevated for the duration of pregnancy until 24–36 hours before parturition when there is an abrupt decline
- detection of this decline in progesterone can therefore also be used to predict the onset of expected parturition
- these techniques work well since progesterone can be easily measured using an ELISA technique in the practice laboratory [1]
- coincidental with the pre-partum decline in progesterone is a decrease in rectal temperature. This pre-partum hypothermia may be detected clinically and be useful for predicting parturition (see 14.4)

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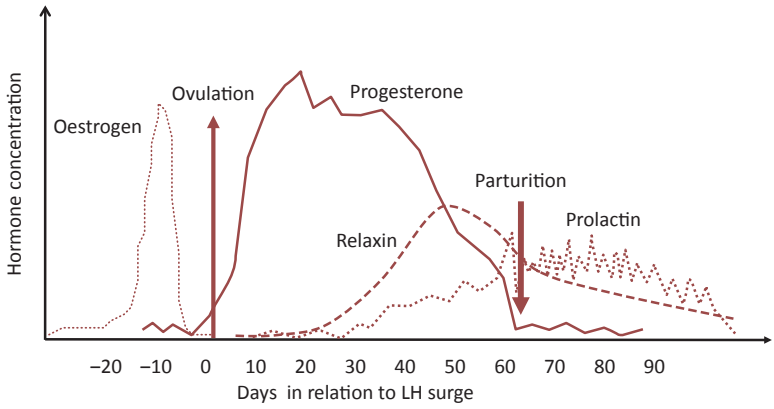


Figure 12.1 Schematic representation of the changes in hormone concentration during pregnancy.

12.2 ENDOCRINE CHANGES IN BLOOD (Figure 12.1)

Progesterone

- progesterone is often described as the 'pregnancy hormone' since its actions appear essential for maintenance of the normal pregnancy. These include closure of the cervix, reduced uterine contractions, endometrial glandular development, increased endometrial glandular secretion and, importantly, enlargement of the mammary glands
- progesterone concentrations are elevated throughout pregnancy
- concentrations of progesterone are however similar during pregnancy to those in non-pregnant bitches (those in normal metoestrus (dioestrus))
- the uterus does not synthesize progesterone as in other species and the corpora lutea are the sole site of production
- in late pregnancy progesterone concentrations are relatively low, but fall sharply when the corpora lutea are lysed 24–36 hours pre-partum [1]

Oestrogen

- the role of oestrogen for much of pregnancy is not certain but it has an important roles in mammary development prior to lactation and relaxation of the reproductive tract prior to parturition
- concentrations are probably the same in pregnancy as in metoestrus (dioestrus); reported differences may reflect measurement of different oestrogens by different assay systems
- oestrogen concentrations increase steadily throughout pregnancy and reach peak values immediately before parturition
- concentrations decrease abruptly to basal after parturition

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Prolactin

- prolactin has several roles in pregnancy, importantly these include stimulation of milk production, but also progesterone is luteotrophic (it stimulates the production of progesterone); indeed it is the most important luteotrophic hormone in the bitch
- concentrations increase in the latter half of pregnancy, as progesterone concentrations decrease (this increase follows the decrease in progesterone and is an attempt to maintain progesterone production since prolactin is luteotrophic)
- similar changes in prolactin occur in non-pregnant bitches and are associated with false pregnancy, although the broad rise in prolactin during the second half of pregnancy appears to be clearly greater than most non-pregnant cycles
- there is a transient large surge during the decline in progesterone 24–36 hours before parturition
- prolactin concentrations are elevated but fluctuate during lactation

Relaxin

- relaxin has an important role in pregnancy which is to ensure that there are changes in the reproductive tract that will allow the passage of the fetuses at the end of pregnancy
- concentrations of relaxin start to increase from the 25th day; this can be used as a pregnancy test and a commercially available kit is commonly used in veterinary practice
- peak values occur at days 40–50 and are followed by a slight decline before parturition
- relaxin is the only known pregnancy-specific hormone in the dog

12.3 DEVELOPMENT OF THE CONCEPTUS

- in the bitch when oocytes are ovulated they are not fully mature (they are ovulated as primary oocytes)
- oocyte maturation with extrusion of the polar body to produce the mature (secondary) oocyte occurs during the 2–3 days after ovulation
- the secondary oocyte (ovum) can be fertilized from 2 days until about 5 days after ovulation
- fertilization of the ovum occurs in the distal Fallopian (uterine) tube
- the fertilized ovum is called the zygote
- rapid division (cleavage) of the zygote produces a morula which is a solid ball of cells
- the morulae enter the uterus from the Fallopian tubes at about 9 days after ovulation, i.e. about 6 days after fertilization

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- a cavity (the blastocoele) develops rapidly in the centre of the morula (which is now composed of about 30 cells) and the resulting fluid-filled sphere is called a blastocyst
- the single-cell-thick wall of the blastocyst will develop into the fetal membranes which contribute to the placenta; a small button of cells to one side of the cavity, the inner cell mass, will mainly give rise to the embryo proper
- until this stage the developing embryo has remained the same size as the original ovum because it has been surrounded by a non-cellular membrane, the zona pellucida; with successive divisions the cells have become smaller
- disappearance of the zona pellucida allows the developing conceptus to increase in size
- the conceptuses grow and arrange themselves in a roughly even manner along the uterine horns, but are not attached to the endometrium until 21 days after ovulation (this is very late compared with many domestic species)
- between the 21st and 40th day, each growing conceptus is roughly spherical
- until the 35th day, there is a portion of constricted uterus between each of these discrete conceptuses (Figure 12.2)
- after 35 days, continued growth of the conceptuses expands the constrictions, so that adjacent chorio-allantoic membranes come into contact with each other and both uterine horns become uniformly distended
- early in pregnancy there is a relatively large amount of fetal fluid (mainly in the yolk sac) compared with the size of the developing puppy (embryo)
- at about 35 days the arrangement of cells within the embryo, i.e. the process of organogenesis, is complete and thereafter the developing individual is called a fetus; its subsequent development is mainly due to an increase in size
- as pregnancy develops, the allantois becomes the main fluid-filled cavity in the conceptus, but the volume of fluid becomes relatively reduced compared with the size of the growing fetus [2]; in the early stages the very delicate embryo requires cushioning (by fluid) from external stimuli; the larger fetus is more robust and needs less 'protection'

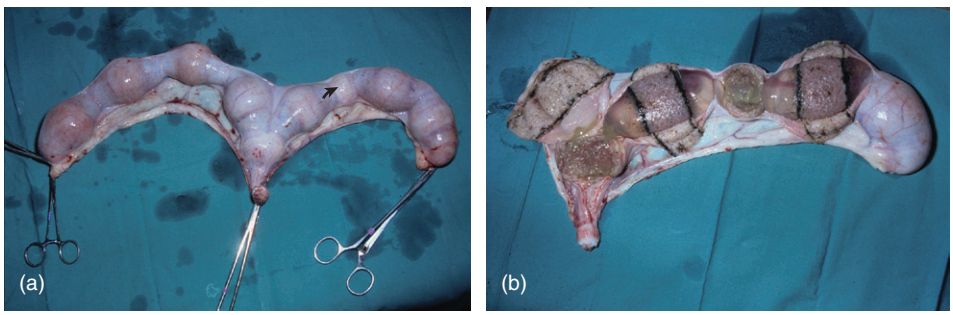


Figure 12.2 Uterus in mid-pregnancy: (a) immediately after ovariohysterectomy; note the small conceptual swelling (arrowed); (b) partial dissection of the uterus; resorption sites can be seen in the body and right horn (shown on the left in a). The zonary nature of the placenta and the marginal haematoma are clearly illustrated.

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- the small amount of fetal fluid that remains at parturition acts as a lubricant during birth

12.4 DEVELOPMENT OF THE PLACENTA

- at around the 21st day after ovulation, a permanent attachment begins to develop between each conceptus and the endometrium; this process in the bitch is best described as nidation (and sometimes wrongly called implantation) and results in the formation of the placenta
- the placenta is the union between the outer set of fetal membranes (the allantochorion) and the lining of the uterus (the endometrium)
- vascular finger-like villi on the surface of the allantochorion fit tightly into cavities (crypts) in the endometrium; exchange of gases and nutrients occurs between the circulatory systems (capillaries) of the dam and fetus in the placentae
- the placenta of the dog is described as being zonary, i.e. there is a band of placental attachment around the centre of each conceptus whilst at the poles there is no placenta (Figure 12.2)
- over most of this placental area the epithelium and connective tissue of the endometrium are lost, so that the villi are in direct contact with maternal capillaries; the placenta is said to be endotheliochorial
- along both edges of the placental zone the maternal blood vessels rupture; the leaked blood is contained along the periphery of the placenta and is called the haemochorial border or marginal haematoma
- the marginal haematomas contain large pools of stagnant maternal blood from which the extra-embryonic circulation absorbs various metabolites, especially iron
- destruction of the blood pigment haemoglobin in this marginal haematoma produces the green uteroverdin; this is seen when placentae start to break down at parturition

12.5 OTHER CHANGES DURING PREGNANCY

A variety of behavioural and morphological changes occur during pregnancy, many progress to parturition.

Behaviour

- early in pregnancy there are no obvious changes in behaviour
- bitches often become anorexic at around the time of nidation but then appetite tends to increase in the latter two-thirds of pregnancy
- in the last one-third of pregnancy the bitch may make a nest and become protective of the nest area; some bitches start to collect toys in the nest area

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- in the last few days before parturition the bitch may seek seclusion
- these behavioural events are not diagnostic of pregnancy since they are commonly seen in non-pregnant bitches with signs of pseudopregnancy (see 19.8)

Appetite

- this is commonly depressed in the first one-third of pregnancy; it does not affect the growth of pups but may alarm the owner
- the requirement for available carbohydrate is increased in later pregnancy and appetite then increases
- during late pregnancy the bitch should be given several small meals a day

Abdominal enlargement

- there is no noticeable change in body conformation until day 45–50
- overfeeding may mimic pregnancy
- the total bodyweight of the bitch may increase 20–55% during gestation
- in the last third of pregnancy the bitch will lose her waist and the abdomen may appear pendulous

Mammary development

- an increase in blood supply may be seen in all bitches, around 25–30 days after ovulation, i.e. the veins around the base of the nipples become more prominent
- mammary gland enlargement is often noticeable from mid-pregnancy onwards (but this is similar in bitches that are not pregnant)
- continued development occurs in the last one-third of pregnancy to produce milk in both pregnancy and pseudopregnancy

Haematology changes

- there are considerable changes in blood profile that occur in pregnancy; these are overlooked in many textbooks
- at approximately 33–37 days of gestation there is an increase in concentrations of acute phase proteins in blood; this may be used as a method of pregnancy diagnosis (see 13.8)
- a progressive anaemia starts during the third week of pregnancy; it appears not to affect the health of the bitch:
  (a) the packed cell volume (PCV) is usually less than 40% at day 35 and below 35% at term
  (b) the major cause of the anaemia is the result of increased plasma volume causing haemodilution
- from 1 week after oestrus there is reduced sensitivity to insulin, but not glucagon, and this may aggravate pre-existing diabetes mellitus

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Mucoid vulval discharge

- this is often present such that the author considers this normal in early pregnancy
- a normal vulval discharge must be distinguished from the pathological discharge due to pyometra, which occurs at the same stage of the cycle (see 16.5)

12.6 PRODUCTION, MAINTENANCE AND EJECTION OF MILK

Lactation

- the production and secretion of milk is called lactation
- initial development of the mammary gland is stimulated by oestrogen when the bitch is in oestrus, and is followed by maturation and further development of the gland during the long luteal phase (of pregnancy or non-pregnancy)
- as progesterone concentrations decrease in late pregnancy there is reduced inhibition on prolactin inhibitory factor and so prolactin is secreted from the anterior pituitary gland
- elevated concentrations of prolactin in the primed mammary gland is responsible for the onset of lactation

Excretion of milk

- the active excretion of milk is stimulated by release of oxytocin from the posterior pituitary gland
- oxytocin causes contraction of myoepithlial cells
- oxytocin release is triggered by either a neurological reflex (stimulation of the nipple) or is conditioned by smells or sounds of the neonates
- other endocrine factors are also involved in milk letdown and these can be inhibited by adrenaline; therefore stressful situations, including surgical intervention at the time of parturition, can result in failure of milk excretion (failure of milk letdown); this can be treated by administration of oxytocin

NB: Failure of milk production (agalactia) cannot be treated by oxytocin administration (see 22.19).

Galactopoiesis

- the maintenance of milk production is termed galactopoiesis
- the process of stimulation of the posterior pituitary gland, oxytocin release, and the other hormonal changes are the mechanism by which milk production is maintained

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- at weaning there is a reduction in sucking by the pups, therefore less oxytocin release and so milk production ultimately ceases

REFERENCES

1 England, G.C.W. and Verstegen, J.P. (1996) Prediction of parturition in the bitch using semi-quantitative ELISA measurement of plasma progesterone concentration. *Veterinary Record* **139**, 496–497.

2 England, G.C.W. (1992) Ultrasound evaluation of pregnancy and spontaneous embryonic resorption in the bitch. *Journal of Small Animal Practice* **33**, 430–436.

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13 Pregnancy Diagnosis

In most domestic species pregnancy interrupts normal cyclical activity by increasing the length of the luteal phase and delaying the return to oestrus. The physiology of the bitch is unlike other species in that the luteal phase is similar in length for both pregnancy and non-pregnancy. In addition, the fertilization period of the bitch differs from that of most other mammals. In particular it should be remembered that:

- in most bitches the day of ovulation is not known and both 'early' and 'late' matings can result in a pregnancy:
  (a) when the interval from ovulation to parturition is calculated this is always 62–64 days; mating on the day of ovulation will result in whelping commencing at the expected time
  (b) matings that occur before ovulation may be fertile should spermatozoa reside within the reproductive tract 'waiting' for ovulation; counting from this 'early' mating to parturition results in an apparently long pregnancy length (up to 72 days from mating to parturition)
  (c) oocytes remain fertilizable for at least 5 days after ovulation; matings that occur after ovulation will result in an apparently short pregnancy length (as short as 58 days from mating to parturition)
- this physiological variation is important for pregnancy diagnosis since:
  (a) bitches that are examined for pregnancy diagnosis after an 'early' mating will appear to have a pregnancy that may not be as advanced as expected; it the worst case scenario a bitch that is examined shortly after mating for pregnancy diagnosis may be falsely diagnosed and not pregnant because the examination was undertaken before a positive diagnosis could be made
  (b) bitches that are examined for pregnancy diagnosis after a 'late' mating will appear to have a pregnancy that may be further advanced than expected
- endocrine changes in the bitch are dissimilar to those of the majority of other domestic species and methods of pregnancy diagnosis cannot simply be adapted

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13.1 ABSENCE OF A RETURN TO OESTRUS

- in the bitch the inter-oestrous interval is almost identical in pregnant and non-pregnant cycles
- an absence of a return to oestrus is not specific for pregnancy

13.2 BEHAVIOURAL CHANGES

- both pregnant and non-pregnant bitches may exhibit behavioural changes typical of pregnancy
- these are usually associated with an increase in plasma prolactin concentration
- food intake may increase in both pregnant and non-pregnant bitches

13.3 PHYSICAL CHANGES

- pregnant bitches commonly have a small volume mucoid vulval discharge approximately 1 month after mating
- body weight begins to increase from day 35 of pregnancy
- abdominal swelling may be noted from day 40 of pregnancy
- mammary gland enlargement is usually obvious from day 35, at which time serous fluid can be expressed from the glands
- colostrum may be present in the teats in the last 7 days of pregnancy

NB: Similar features are common in pseudopregnant bitches.

13.4 ABDOMINAL PALPATION

- can be highly accurate if performed at an appropriate stage of pregnancy
- may be difficult in large, obese or nervous animals
- may be inaccurate if the bitch was mated 'early' such that pregnancy is not as advanced as anticipated

Technique

Small dogs

- one hand can be used for abdominal palpation
- abdominal contents are then allowed to pass between the fingers and thumb as the hand is drawn ventrally

Large dogs

- a two-handed technique is preferable
- ideally the owner and clinician should be seated and facing each other

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- the owner holds the bitch's head
- then the clinician's extended hands are placed on either side of the abdomen, midway between the anterior pelvis and last rib
- both hands simultaneously compress the abdomen and are then moved dorsally so that viscera slip between the fingers

Interpretation

- the optimum time for diagnosis is approximately 1 month after mating
  (a) the conceptuses are spherical in outline, and may vary between 15 and 30 mm in diameter
  (b) they are tense fluid-filled structures and can be readily palpated
- from day 35 the conceptuses become elongated, enlarged and tend to lose their tenseness; they may be less easy to palpate at this time
- after day 45 the uterine horns tend to fold upon themselves, resulting in the caudal portion of each horn being positioned against the ventral abdominal wall, and the cranial portion of the same horn being positioned dorsally
- after day 55 the fetuses can often be identified, especially if the forequarters of the bitch are elevated and the uterus is manipulated caudally towards the pelvis
- it is difficult to accurately count the number of conceptuses by palpation except when performing an early examination

13.5 IDENTIFICATION OF FETAL HEART BEATS

- in late pregnancy it is possible to auscultate fetal heart beats using a stethoscope
- fetal hearts may also be detected by recording a fetal electrocardiogram
- Doppler ultrasound machines (commonly used for pregnancy diagnosis in sows) can be used for detection of fetal heart beats
- all three methods are diagnostic of pregnancy but none are commonly used
- fetal heart beats are simple to detect because the heart rate is usually more than twice that of the dam

13.6 RADIOGRAPHY

- uterine enlargement can be detected from day 30
- at this stage the enlarged uterus can be readily identified in the caudal abdomen, originating dorsal to the bladder and ventral to the rectum; it frequently produces cranial displacement of the small intestine
- the early pregnant uterus has only soft tissue opacity and cannot be differentiated from other causes of uterine enlargement, such as pyometra
- correct pregnancy diagnosis is not possible until after day 45 when mineralization of the fetal skeleton is detectable

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- progressive mineralization results in an increasing number of bones that can be identified (Figure 13.1)
- it is unlikely that the fetuses will be damaged by the ionizing radiation after day 45; however,
  (a) earlier examination during organogenesis is potentially hazardous
  (b) sedation or anaesthesia of the dam may be required and is a potential risk
- in late pregnancy the number of pups can be reliably estimated by counting the number of fetal skulls

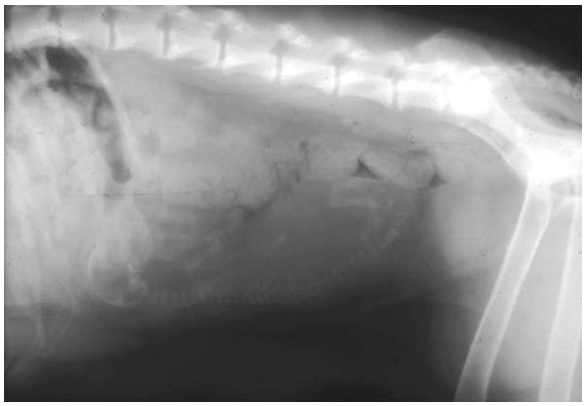


Figure 13.1 Lateral radiograph of the abdomen of a pregnant bitch. The mineralized fetal skeleton can be seen (a skull is easily visible overlying the last rib) (courtesy of Marco Russo).

13.7 ENDOCRINOLOGICAL TESTS (Figure 13.2)

- plasma (serum) concentrations of progesterone are not useful for the diagnosis of pregnancy
- pregnancy-specific changes in plasma and urine oestrogen concentration have not been adequately evaluated
- a significant elevation of plasma prolactin occurs in pregnant bitches compared with non-pregnant bitches but this method has not been adequately evaluated

Relaxin

- measurement of the hormone relaxin is diagnostic of pregnancy and an enzyme-linked immunosorbent assay is commercially available which can be run in the practice laboratory; this method is simple, rapid and reliable and is suitable when the practice does not have a diagnostic ultrasound machine
- relaxin is produced by the placenta and there is some evidence that plasma concentrations are higher in bitches with greater number of fetuses (there is more placental tissue)

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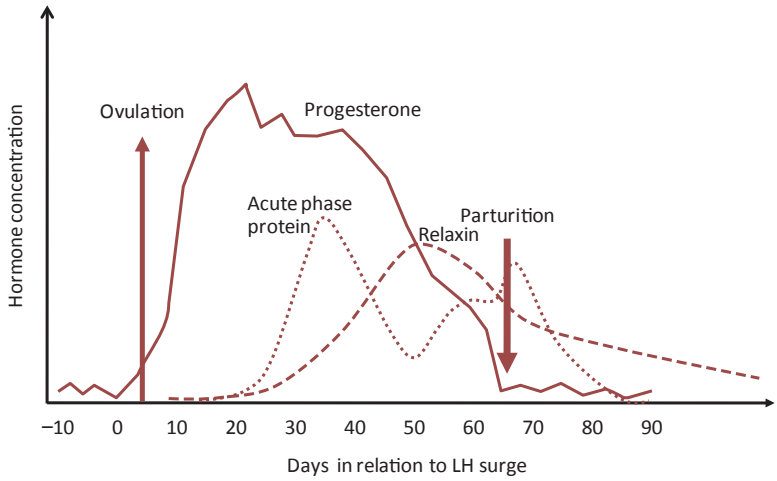


Figure 13.2 Schematic representation of the changes in plasma relaxin and acute phase proteins in the pregnant bitch. NB: Progesterone is not diagnostic for pregnancy since values are not sufficiently different between pregnant and non-pregnant bitches.

13.8 ACUTE PHASE PROTEINS (Figure 13.2)

- an acute phase response occurs in pregnant bitches at approximately the time of implantation
- this response appears to be unique to the bitch
- fibrinogen, C-reactive protein or other acute phase proteins are all sensitive markers of pregnancy
- the initial rise in these proteins occurs from day 20 onwards with a peak at approximately day 40
- false positive diagnoses may result from inflammatory conditions (such as pyometra which occurs at the same stage of the oestrous cycle)
- the rise in fibrinogen concentration is now the basis of a commercial pregnancy test

13.9 ULTRASOUND EXAMINATION (see also 5.8)

When planning diagnosis of pregnancy it is important to consider the variability between the day of mating and the time of ovulation (see 11.1) which may result in a pregnancy being more or less advanced than anticipated. The author prefers to conduct an examination 1 month after the last mating at which time the conceptuses are usually aged between 22 and 33 days from ovulation; at this stage an absence of conceptuses on ultrasound examination is diagnostic of non-pregnancy.

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Early pregnancy (Figure 13.3)

- work in the author's clinic was preeminent for the diagnosis of early pregnancy and estimation of fetal number [1]
- uterine enlargement occurs during the luteal phase whether the bitch is pregnant or non-pregnant
- yolk sac fluid accumulates within the yolk sac and it is not until the yolk sac is filled with a sufficient volume of fluid that the pregnancy can first be imaged
- the anechoic (black appearing) yolk sac can first imaged from 15 days after ovulation (remember mating may not coincide with ovulation)
- at 15 days the anechoic yolk sacs are approximately 2 mm in diameter and are spherical in appearance
- at this stage of early pregnancy the embryo is located adjacent to the uterine wall and is not imaged
- the conceptus rapidly increases in size and may lose its spherical outline, becoming oblate (rugby ball shaped) in appearance

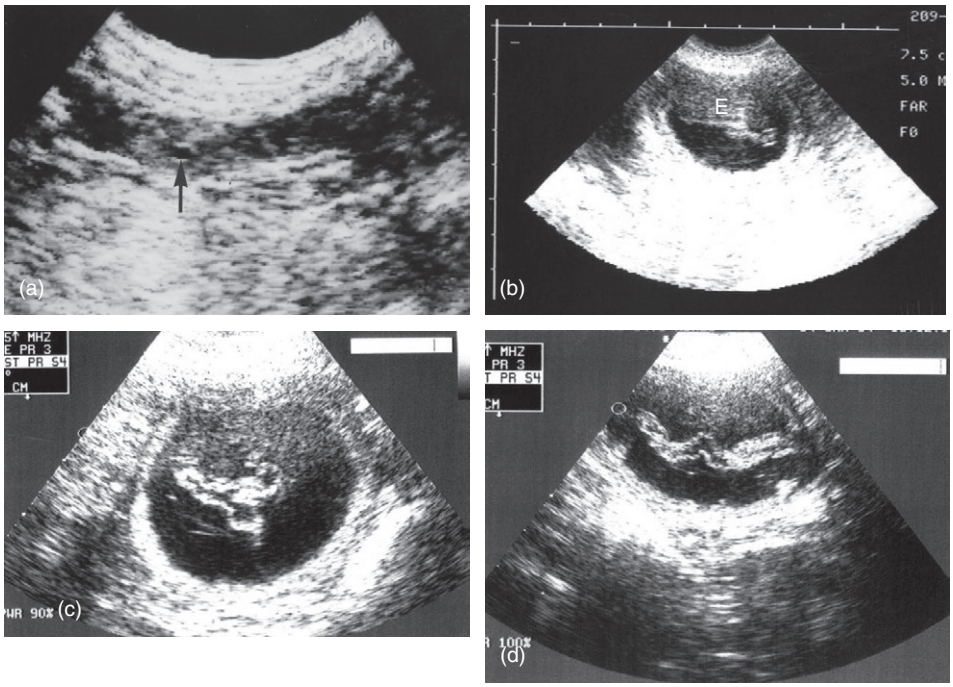


Figure 13.3 Ultrasound images during early pregnancy: (a) fluid-filled conceptus (arrow) within one uterine horn 21 days after ovulation; (b) larger fluid-filled conceptus containing an embryo (E) and developing embryonic membranes; (c) conceptus 30 days after ovulation, demonstrating the corrugated appearance of the collapsing yolk sac in transverse section; (d) the same conceptus at 30 days after ovulation, demonstrating the yolk sac in longitudinal section. 7.5 MHz transducer, scale in centimetres.

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- from day 20 after ovulation the conceptus is approximately 7 mm in diameter and 15 mm in length, and the embryo can be imaged
- the presence of the embryonic heart beat can be detected from approximately 22 days after ovulation
- the developing allantois initially appears as a nearly spherical structure within the conceptus which subsequently increases in size and surrounds the yolk sac
- a third fluid-filled sac, the amnion, may be noted later in pregnancy since it surrounds, and initially is in close apposition to, the fetus

Mid pregnancy (Figure 13.4)

- the most rapid growth of the fetus occurs between days 32 and 55; during this time the limb buds become obvious and there is clear differentiation of the head, trunk and abdomen
- work in the author's clinic was the first to develop a complex measurement model for the assessment of canine pregnancy, estimation of fetal age and prediction of parturition [2]; these data are now widely used in clinical practice – many ultrasound machines have the data programmed into function menus to enable calculation of gestational age during the examination)
- the zonary placenta can usually be easily identified from this stage of pregnancy onwards
- the fetal skeleton becomes evident from day 40 onwards when fetal bone appears hyperechoic, and casts acoustical shadows
- the heart can now be easily identified and large arteries and veins can be seen cranially and caudally
- lung tissue surrounding the heart is hyperechoic with respect to the liver
- from 45 days onwards it is possible to identify the fluid-filled (anechoic) stomach, and a few days later the bladder can be imaged

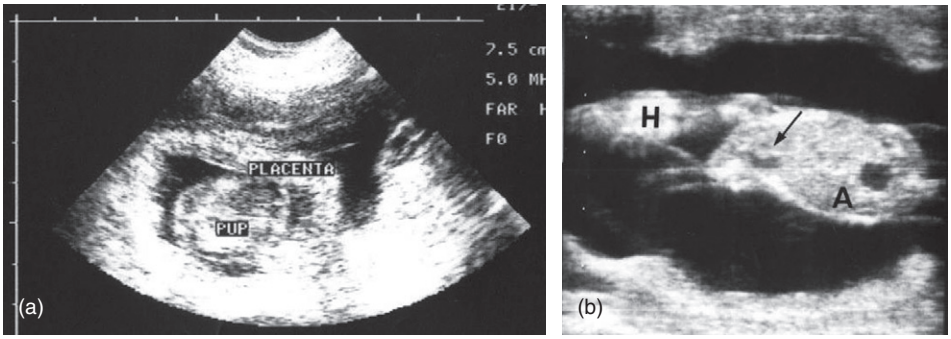


Figure 13.4 Ultrasound images during mid pregnancy: (a) a single fetus surrounded by a small volume of fluid imaged in transverse section; (b) a single fetus imaged in longitudinal section demonstrating the fetal head (H), thorax containing the heart (arrow), and abdomen (A) containing the anechoic bladder. 5.0 MHz transducer, scale in centimetres.

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Late pregnancy (Figure 13.5) [3]

- the head, spinal column and ribs produce intense reflections of the ultrasound and are easily identifiable as bright white regions (sometimes casting an acoustic shadow)
- in the last 20 days of gestation the kidneys can be seen
- the small intestine may be detected
- at this stage the fetus is highly mobile, and limb and whole body movements occur frequently
- swallowing movements can be observed
- hiccupping may occasionally be noticed
- it is difficult to examine more than a few fetuses at this time without clipping an excessive amount of hair

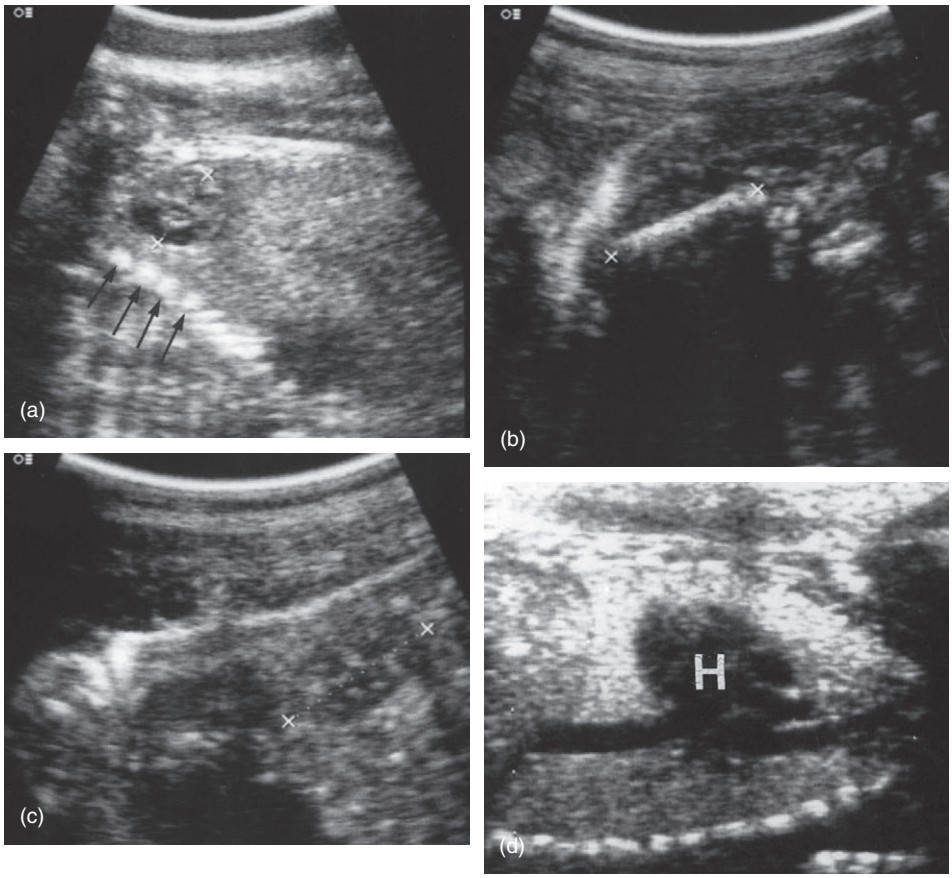


Figure 13.5 Ultrasound images during late pregnancy: (a) the thorax containing the heart (crosses) surrounded by the lungs; the ribs are clearly visible (arrows); (b) a fetal femur (arrows) with distal acoustic shadowing; (c) a fetal kidney (crosses); (d) the fetal heart (H) with great vessels, surrounding lung, liver and diaphragm clearly visible. 5.0 MHz transducer, scale in centimetres.

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Assessment of fetal number [1]

- the accuracy of detecting absolute fetal number is poor
- the greatest accuracy is at the first examination 1 month after mating
- the lowest accuracy is in late pregnancy
- most commonly the number of fetuses is underestimated
- the accuracy is reduced in large litters

Errors with real-time ultrasonography [1,2]

False positive

- such scans are unlikely although empty loops of small intestine and the caudal pole of the kidney may confuse the inexperienced operator
- resorption of several conceptuses has been seen and may account for the absence of puppies after a positive scan

False negative

- failure to detect early pregnancies may occur because:
  (a) the pregnancy may be less than 23 days from ovulation when the examination is performed (this is not likely if the examination is undertaken 1 month after the last mating)
  (b) in small litters the conceptuses may be obscured by intestine (gas)

Overestimation of fetal numbers

- this may occur because of:
  (a) subsequent resorption (most common) [4]
  (b) counting the same conceptus twice

Underestimation of fetal numbers

- this is very likely, particularly in litters of greater than four

REFERENCES

1 England, G.C.W. and Allen, W.E. (1990) Studies on canine pregnancy using B-mode ultrasound; diagnosis of early pregnancy and the number of conceptuses. *Journal of Small Animal Practice* **31**, 321–323.
2 England, G.C.W., Allen, W.E. and Porter, D.J. (1990) Studies on canine pregnancy using B-mode ultrasound; development of the conceptus and determination of gestational age. *Journal of Small Animal Practice* **31**, 324–329.
3 England, G.C.W. (1992) Ultrasound evaluation of pregnancy and spontaneous embryonic resorption in the bitch. *Journal of Small Animal Practice* **33**, 430–436.
4 England, G.C.W. and Russo, M. (2006) Ultrasonographic characteristics of early pregnancy failure in bitches. *Theriogenology* **66**, 1694–1698.

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14 Normal Whelping

14.1 ENDOCRINE CONTROL

- the duration of whelping (parturition) is usually quite protracted in the bitch and its endocrine control is poorly understood
- the trigger for the timing of parturition is likely to involve the maturation of the fetal pituitary–adrenal axis at the completion of fetal development
- maternal cortisol concentrations rise just before parturition but their role is not fully understood
- oestrogens, oxytocin and relaxin are probably involved in the initiation of parturition, but the most likely trigger is prostaglandin which increases in blood 36 hours pre-partum and probably causes luteolysis and the precipitous decline in progesterone
- prior to this progesterone concentrations have been slowly declining throughout the latter part of pregnancy whist oestrogen concentrations are maintained or slightly increase
- a precipitous decrease in progesterone is the final trigger for a change in oestrogen : progesterone ratio which is the major endocrine event associated with the initiation of parturition
- the decrease in progesterone generally occurs 24–36 hours before the onset of parturition
- the decrease in progesterone correlates with a fall in body temperature (progesterone is thermogenic and its withdrawal results in a decrease in temperature); this 'pre-partum hypothermia' generally occurs approximately 24 hours before the onset of parturition
- prolactin concentrations rise during the latter part of pregnancy and peak during lactation; prolactin is probably not involved in the initiation of parturition
- changes in ionic composition of milk occur in several species and can be used to predict parturition; in the author's clinic we found similar changes occurred in bitches' milk but only after parturition so these are not clinically useful [1]

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Figure 14.1 Wooden design whelping box with hinged side to allow pups access to the room when they are old enough.

14.2 PREPARATION OF THE ENVIRONMENT (Figure 14.1)

- careful acclimatization and familiarization of the bitch with the area that she is expected to use for whelping is sensible in the last 2 weeks before parturition is expected
- there is no single perfect whelping area but the following should be considered:
  - (a) there should be human comforts including a chair, hot and cold water and telephone access with important telephone numbers
  - (b) the room is best isolated by a baby gate and door
  - (c) the area should be clean and be capable of being kept clean
  - (d) preferably a separate whelping box should be used with a rail on the inside to prevent pups from being squashed
  - (e) the box should be well constructed to avoid drafts as these can cause chilling of the neonates
  - (f) the sides of the box should be low enough to facilitate the bitch getting away from the pups when she wants to, alternatively the area may have a wall-mounted shelf that the bitch can climb onto but the puppies cannot
  - (g) it is helpful if one side is removable so that when pups are old enough they can be allowed access into the main room
  - (h) the box should be large enough to allow the bitch to be fully extended in any direction which she lies down

NB: It is critical that infectious diseases are not introduced into the whelping area; these are most likely to be introduced on the shoes of visitors, or when visitors handle pups after touching other dogs. Use of overshoes, hand washing and alcohol hand gel should all be considered for visitors.

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Temperature

- artificial heat may be provided, but the bitch should be a good source of warmth
- heat may be provided by a hot water bottle wrapped in a towel, via floor heating or commonly via heat lamps
- initially a temperature of about 26 °C is ideal, but if overhead heating is supplied, ensure that the bitch cannot burn (she will be closer to the lamp than the pups)

Bedding

- provision of material which is easily removed and disposed of when soiled (e.g. fleecy bedding)
- a thick bed of newspaper is ideal as the top layers can be removed as necessary

Quietness

- the whelping box should be in the corner of a room or, better still, in a separate room
- this prevents the bitch from being unwittingly disturbed during whelping
- do not invite the neighbours in to watch

14.3 EQUIPMENT

- it is sensible that the whelping area is stocked with equipment that will be needed or may be needed during a normal but assisted delivery, this will save time and stress at a later date

General equipment

- newpaper and clean bedding
- plastic sacks for disposal of bedding
- mop, bucket and disinfectant
- sealable bags for disposal of clinical waste, e.g. placentae or stillborn pups

NB: Do not dispose of dead pups or placenta; they should be saved in case histological examination is needed (do not put them in the deepfreeze).

Equipment for the bitch

- thermometer and chart for recording rectal temperature
- clock and chart for recording timing of contractions, times of delivery

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- lubricant
- sterile or clean plastic gloves

Equipment for the pups

- scissors and cotton thread for umbilical cords
- dilute disinfectant
- soft towels
- nail varnish or marker pens to enable identification of pups
- syringes or bottles for feeding (including sterilization tablets)
- frozen or commercially available colostrum or knowledge of where to source

14.4 PREPARATION OF THE BITCH

Before mating

- ensure routine vaccination status is up-to-date so that there are adequate antibodies present in the bitch's colostrum
- ensure that the bitch is treated for endoparasites and ectoparasites
- ensure that any gingivitis is treated (by descaling teeth if necessary)

At the time of mating

- if infectious disease is present in the dog or bitch consider artificial insemination if this is allowed
- any other disease of a possibly infectious nature should be treated
- vaccination and parasite treatments can still be administered if this was not done previously, but the window of time shortly after mating (during organogenesis should be avoided)
- consider the use of canine herpes virus vaccination if there is concern about possible exposure; this vaccine is normally given anytime the bitch is in oestrus until 7 days after mating, with a second dose administered at approximately day 55 of pregnancy

Treatments during pregnancy

- the author prefers to administer no drugs during pregnancy if at all possible; lists of therapeutic agents with minimal effect upon the developing embryo and fetus are available and should be consulted before any drug is administered
- drug administration should, however, be avoided during organogenesis if possible
- the bitch can be treated for endoparasites (note that prolonged courses of the newer anthelmintics, fenbendazole and oxfendazole, given in later pregnancy can be used to kill migrating larvae)

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Late pregnancy

- check for fleas and ear mites
- do not force extra food on the bitch; it is rarely necessary to increase the volume of food before about 40 days of pregnancy
- feed small amounts and often
- avoid calcium supplementation; this may depress parathyroid function and prevent mobilization of calcium at parturition leading to inertia (see 22.1) or eclampsia (see 22.15)
- introduce the bitch to the whelping area at least 2 weeks before expected parturition and get her used to sleeping there
- monitor mammary development in the last week; if this appears inadequate, or if nipple numbers are low compared with the expected size of the litter, have milk supplements on hand
- in long-haired breeds it is advisable to cut hair away from the mammary glands to make the nipples more accessible and from the perineum and back legs to make cleaning the bitch easier after whelping

Temperature monitoring

- start taking the bitch's rectal temperature about 1 week before the expected whelping date
- temperature should be taken at least three times a day
- normal temperature is about 38 °C (100.5 °F) during late pregnancy
- a fall below 37.5 °C (99.5 °F) indicates that parturition should start within 24 hours (Figure 14.2)
- the temperature drop may only be transient, but if temperature is taken three times daily, a single low reading may be due to incorrect procedure
- if no signs of whelping occur within 48 hours after the temperature fall, the bitch may be suffering from primary uterine inertia (see 22.1) and may require a caesarean operation
- temperature monitoring is essential in bitches known to be carrying small (one or two pups) or large litters, as these are most prone to inertia

Progesterone monitoring

- in normal whelpings this is often unnecessary
- however when there is concern about when parturition is due the measurement of plasma progesterone is very useful since this decreases just before the onset of parturition
- in the author's clinic we have shown that simple ELISA techniques can be used to accurately predict the onset of parturition [2]; these findings have wide clinical applications in monitoring of normal and suspected abnormal whelpings (Figure 14.2)

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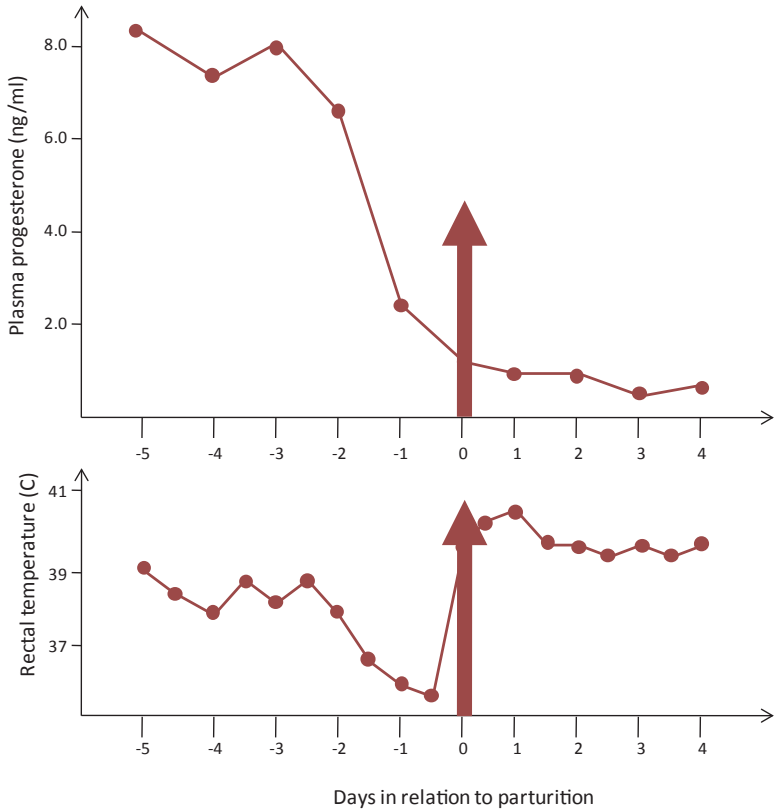


Figure 14.2 Changes in plasma progesterone and rectal temperature at the time of parturition.

NB: Progesterone monitoring during oestrus can also be used to predict the onset of parturition which occurs approximately 61 days after high progesterone has been detected.

14.5 THE 'OVERDUE' BITCH

- pregnancy length is remarkably consistent at 62–64 days from ovulation
- however, when measured from first or last matings, pregnancy lengths can be very variable (Figure 14.3)
- longer than expected pregnancies can be due to:
  - (a) bitch being mated several days before ovulation; she can still become pregnant and pregnancy length can be 72 days from mating until the onset of parturition
  - (b) bitch not being pregnant! – this can be investigated by radiographic or ultrasound examination (see 13.6 and 13.9)

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(c) mating dates being incorrect
(d) pregnancy length being miscalculated
(e) primary uterine inertia (see 22.1)

NB: Shorter than expected pregnancies can be due to a bitch being mated several days after ovulation; she can still become pregnant and the pregnancy length can be 56 days from mating until the onset of parturition.

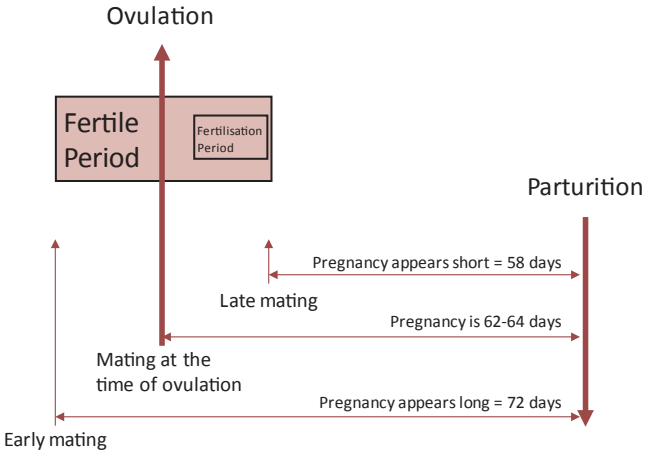


Figure 14.3 Schematic representation of the length of pregnancy in the bitch in relation to mating early or late during the fertile period.

14.6 FIRST STAGE PARTURITION

- for 2–3 days before parturition bitches are often restless, seek seclusion and eat less food, possibly in response to elevated prolactin concentrations
- the beginning of first stage cannot be accurately recognized unless the bitch is carefully monitored
- during this time, which is the preparation for the expulsive (second) stage, there are uterine contractions; these can sometimes be seen if the bitch is lying in lateral recumbency, otherwise a monitoring device is required to measure uterine activity (tochodynomometery)
- it is important to differentiate uterine contractions (onset of first stage) from abdominal contractions (onset of second stage); this is easy using a tochodynomometer where uterine contractions are measured in mmHg
- uterine contractions initially are not very frequent and the cervix is closed; some bitches are more upset by these contractions than others
- the onset of uterine contractions is closely related to the fall in circulating progesterone concentration

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- gradually the contractions appear to become more intense and frequent as a pup is being pushed towards the dilating cervix
- as first stage parturition progresses there is a gradual increase in the uterine contractions; these increase from one short-duration contraction per hour to approximately one contraction every 10 minutes
- the time at which the cervix dilates fully cannot be ascertained because the bitch's vagina is relatively long and the cervix is unlikely to be reached by digital examination
- accurate examination of cervical patency requires endoscopic examination
- the allantochorion of the first pup ruptures in the vagina; the presence of a pup and its fluid and membranes in the vagina usually stimulates abdominal straining, although some pups are born with very little evidence of this
- the beginning of straining, or the appearance of fetal fluid or a pup at the vulva, marks the change from first to second stage parturition
- uterine contractions during first stage parturition may cause:
  (a) restlessness
  (b) anorexia
  (c) panting and a 'worried look'
  (d) shivering
  (e) nesting behaviour
  (f) looking at the flanks
  (g) vulval licking
  (h) vomiting if recently fed
  (i) swelling and relaxation of the vulva
  (j) appearance of mucus at the vulva
  (k) abdominal relaxation which allows easier palpation of the fetuses

NB: The length of first stage parturition (0–36 hours), intensity and behavioural characteristics are very variable, even in successive pregnancies in the same bitch.

There is usually milk in the mammary glands throughout first stage parturition.

14.7 SECOND STAGE PARTURITION

- uterine contractions increase in frequency and amplitude and when using a tochodynomometer abdominal contractions can be seen superimposed on top; these are of longer duration and lower amplitude than the uterine contractions
- in normal second stage there is continuation of uterine contractions in the background throughout parturition; infrequent and low-amplitude uterine contractions are seen in bitches with abnormal parturition
- usually the amnion (water bag) of the first pup is seen at the vulva soon after abdominal straining begins
- passage of the head through the vulva may cause signs of pain in primigravida, but the pup is usually born with little effort

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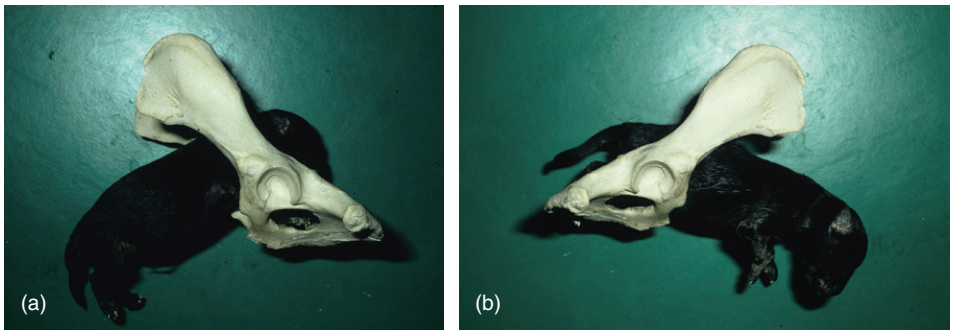


Figure 14.4 Normal presentation position and posture in the dog fetus. The most common orientation is anterior longitudinal presentation, in dorsal position with the head and limbs extended rostrally. The pups move upwards to the pelvic inlet (a) and then downwards through the vagina, vestibule and vulva (b).

- pups can only be born normally in longitudinal presentation, i.e. the long axis of the puppy is parallel to the long axis of the bitch (see 22.5)
- anterior presentation (with the pup's head born first) is most common, but posterior presentations occur so frequently as to be accepted as normal; however, obstructive dystocia may be caused by posterior presentation of the first pup
- the position of the pup during normal birth is dorsal, i.e. the back of the pup is uppermost, towards the back of the dam; this allows the natural curve of the pup's spine to correspond with the curved course it must take – initially upwards into the bitch's pelvis then downwards through the vulva (Figure 14.4)
- the posture of the pup is a description of the disposition of its head and legs; the legs may be either extended or flexed during normal parturition, but in anterior presentation the head and neck must be extended, i.e. the nose is leading, or obstruction will occur
- a breach birth is a posterior presentation with the hind limbs flexed, i.e. the feet are extended forward and the tail is seen first
- after the birth of the pup the bitch will lick and rupture the amnion, and rupture the umbilical cord; further licking helps to dry and stimulate the pup

NB: It is important to ensure that excess nibbling, especially at the umbilicus, does not occur; this may result in an umbilical hernia or the bitch eating part of the pup.

- the allantochorion (see 12.4) (placenta or afterbirth) may be expelled at a variable time after the pup, and some pups are born within it; a second pup may follow before the expulsion of the first allantochorion
- the bitch will usually attempt to eat the allantochorion; it is not necessary that she does so and it will probably cause her to develop diarrhoea
- the interval between the delivery of successive pups is very variable
- delivery from each horn is usually alternate

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- it is often difficult to decide when a problem has arisen; as a rough guide unproductive straining for 30 minutes may indicate an obstruction, although a normal pup may be born after this time
- if more pups are expected and the bitch is restless, will not let the pups suck for long, but is not straining, 2–3 hours may elapse without trouble ensuing
- longer periods (up to 6 hours) may occur between successive pups with the bitch showing no signs of discomfort

14.8 THIRD STAGE PARTURITION

- this terminology is 'borrowed' from other species where classically third stage parturition refers to the passage of the allantochorion after second stage parturition is complete
- in the bitch this process occurs throughout the second stage and cannot be simply defined
- however, involution of the empty uterus could be said to constitute a third stage, although its duration would be hard to define

14.9 INDUCTION OF PARTURITION

- there are few indications for induction of parturition in the bitch, although on occasion it may be warranted when there is inter-current disease affecting the bitch
- there are reports of using pharmacological induction of parturition to achieve whelping at a specific time when additional human assistance may be arranged
- overall induction of parturition to a reliable time is only moderately successful and should only be attempted when the bitch has reached 95% of normal pregnancy length and when constant supervision can be provided after drug administration
- the most effective regimes appear to be the administration of progesterone receptor antagonists (e.g. aglepristone) followed by repeated does of oxytocin
- administration of long duration corticosteroids have been said to initiate parturition, but since they are given at a time when whelping is expected anyway, assessment of results is difficult
- oxytocin may stimulate uterine contractions in cases of primary uterine inertia if it is felt sure that the cervix has relaxed; this can only be evaluated adequately by endoscopic examination

14.10 CARE OF THE BITCH AFTER PARTURITION

- at the end of parturition the bitch will be tired and her perineum will be soiled
- she should be offered water to drink and gross contamination should be washed away; after an hour or so when the pups are settled she should be

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moved out of the whelping box but allowed to see the pups whilst her perineum is cleaned and dried
- for the first few days the bitch may not wish to leave the pups and so she should be offered food and water whilst in the whelping box, but she should be taken out regularly to urinate and defecate; exercise is important to encourage reduction in size of the uterus and pushing out of uterine fluid
- some maiden bitches may not adequately stimulate the pups to urinate and defecate, so she should be encouraged to lick the pups to do this; some advocate application of a little honey to the pup's perineum

REFERENCES

1 England, G.C.W. and Verstegen, J.P. (1997) Progestogen concentration and ionic composition of the mammary secretion of periparturient bitches. *Journal of Reproduction and Fertility* **51**(Supplement), 209–214.
2 England, G.C.W. and Verstegen, J.P. (1996) Prediction of parturition in the bitch using semi-quantitative ELISA measurement of plasma progesterone concentration. *Veterinary Record* **139**, 496–497.

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15 Care of Neonatal Puppies

15.1 PHYSIOLOGY AND HUSBANDRY

- immediately after birth puppies have immature physiological responses when compared with adults:
  (a) they have limited ability to regulate or control body temperature
  (b) they have poor control of fluid balance or energy levels
  (c) their immune system is not completely developed
  (d) they may display irregular respiratory patterns
- each of these affect their susceptibility and response to disease and this results in a high mortality rate seen in commercial kennels (10–30%), especially within the first 3 weeks of life
- importantly, however, mortality rates can be significantly reduced with appropriate monitoring and care
- after birth pups are entirely reliant upon the dam; they are born with their eyes and external ear canals closed, they have limited smell and relatively low myelination of nerve fibres
- the eyes begin to open at 10–14 days and the external ear canal at 14–16 days, with cells sloughing off from the ear canal for 1 week after the canal opens
- pups have limited locomotion ability – restricted to simple 'rooting' behaviour (a scrabbling of the fore limbs to provide forward motion and a circular movement of the head in attempt to locate a nipple) which can result in them becoming stuck in corners unless moved by the dam

15.2 BEHAVIOUR

- the early behavioural periods of the pup are closely linked to the underlying physiology; the periods are defined as (1) neonatal, and (2) transitional, and these precede the socialization period (see 1.1).

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The neonatal period

- this extends from birth until the pups open their eyes
- during this time the pup is predominantly sleeping, whilst sucking and voiding account for only 10–20% of time
- the pup is totally dependent upon the dam and can react to stimuli (such as warmth) but do so only by the rooting response noted above
- voiding of faeces and urine only occurs following stimulation of the perineum by the dam licking this area

The transitional period

- extends from approximately 10 days until 4 weeks of age
- there is progressive myelination of the nervous system, the pups can now experience pain, see, hear, and progressively they are able to move their hind limbs
- locomotion ability increases and the pups start to explore the nest, follow the dam and play with each other
- there are increased periods of wakefulness which can account for 40% of time

15.3 APPEARANCE OF THE NORMAL HEALTHY NEONATAL PUP (Figure 15.1)

- has a round and full abdomen and skin that is warm and elastic
- sucks enthusiastically for a short period approximately every 2–3 hours
- birth weight should increase by 5–10% per day; rate of weight gain has been linked to survival, and daily weight gain should be monitored to allow quick identification of any puppies who are at risk
- spend approximately 80% of their time sleeping with an activated sleep pattern (twitching movements when asleep)



Figure 15.1 Normal pups have a rounded abdomen, suck enthusiastically and are lively at feeding times but otherwise are asleep.

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- only cry when hungry or disturbed
- have a body temperature of 35–37 °C in the first few days and 38 °C thereafter
- have an irregular respiratory rate: 10–20 breaths per minute which increases after the first few days
- have a high heart rate: 150–220 beats per minute which increases after the first few days

15.4 CARE OF NEONATES AFTER PARTURITION

- immediately after parturition the dam should sever the umbilical cord and lick the puppy to remove the fetal membranes; this encourages respiration and warms and dries the pup
- the bitch should then encourage the pup to move to the teat and suck
- assistance may be necessary where the dam does not or cannot exhibit these behaviours and can include:
  - (a) removal of fetal membranes and fluid from the mouth and nose using a dry towel, bulb syringe or pipette
  - (b) encouraging fluid to drain away by holding the puppy cupped in the hand with the head tilted downward and hind quarters elevated whilst supporting the head and neck (pups should not be swung to clear the airway as this can result in brain trauma)
  - (c) rubbing the puppy briskly to help stimulate breathing and to warm and dry the pup
  - (d) artificial respiration if there are less than 10 breaths per minute; use of oxygen via intubation or tracheotomy can be very helpful
  - (f) clamping and severing the umbilical cord 1 cm from the abdomen and treating with diluted chlorhexidine

Clinical examination

- once the puppies are stabilized they should be sexed, weighed and checked for congenital abnormalities such as anal atresia, limb deformities, cleft palate and harelip, which can affect between 1% and 15% of newborn puppies depending on the breed

Reducing hypothermia

- pups are wet after birth and at risk of hypothermia; this risk can be reduced by adequate drying, ensuring energy intake, providing a proper nest area, encouraging huddling and providing external heat sources
- even in ideal situations it can take 5 hours before their body temperature returns to normal (Figure 15.2)

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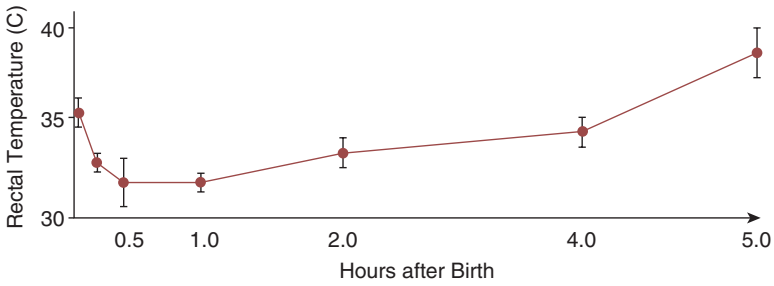


Figure 15.2 Pups rapidly lose heat after birth and it can be 5 hours before they return to a normal body temperature; it is critical all is done during this time to avoid additional heat loss.

- the ideal environment is a temperature of 30–33 °C in the first 24 hours, 26–28 °C in the first week and lower temperatures thereafter; it is helpful for the nest area to be controlled at this temperature but for there to be cooler areas that the bitch can escape to
- bedding should be changed frequently to ensure it is clean and dry

Ensuring energy intake

- pups are born with low glycogen reserves and poor body fat reserves with little ability to generate energy and so they can be predisposed to hypoglycaemia in the first 15 days of life
- the first milk, called colostrum, provides high levels of antibodies and energy
- ensuring that puppies suck soon after birth can help prevent hypoglycaemia, especially following hypothermia or hypoxia
- the availability of teats, poor milk production, an inexperienced, difficult or ill dam, weak pups or continual disturbance by humans can all affect food intake
- alternative sources of colostrum, including commercially available products, should be provided in these cases

Ensuring normal growth and identifying sick pups

- pups that are normal grow at a rapid and predictable rate; this can easily be monitored by assessing the weight of the pups on a daily basis
- daily weighing requires that each pup is uniquely identifiable and use of marks placed on ears or different places on the tail using nail varnish or marker pens facilitates this
- daily weighing seems onerous but is the best way to identify pups that are not feeding well and are predisposed to becoming ill

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Ensuring voiding

- until puppies are 3 weeks old there is no voluntary control of urination or defecation and this is stimulated by the dam licking the perineal area
- the dam should be observed to ensure she is fulfilling this function

Preventative healthcare

- should have included immunization of bitches before breeding or during pregnancy (for canine herpes virus) to provide passive immunity in colostrum
- de-worming programme should start at 3 weeks of age and progress after weaning (see 1.2)

Artificial rearing

- may be required in large litters where the dam has insufficient teats or milk, where there is inadequate milk production, where the dam is a poor mother or due to death of the dam
- in some cases it is possible to foster some or all pups to another bitch that has lost her pups or has a small litter
- pups in large litters may be rotated between artificial rearing and being reared by the dam, allowing them to stay in the nest which will allow adequate socialization
- in cases where the dam dies during birth, colostrum can be expressed from the bitch or canine colostrum from a commercially available or frozen source should be administered
- commercially available milk replacements are advised when the mother's milk is inadequate or unavailable and should be fed at a temperature of 37–38 °C; the total volume to be fed is based on puppy weight and growth and be evenly divided across feedings every 3 hours in the first week with the interval gradually increased to every 6–8 hours by 4 weeks of age
- if puppies will not suck, milk can be administered via orogastric tube or feeding bottle; all food should be fed at the appropriate temperature, best measured with a thermometer and not by sprinkling on the back of a hand

Bottle feeding

- commercially available bottles and teats are preferred; they are more efficient and introduce less gas into the stomach than trying to feed from a syringe or using a dropper bottle
- bottles should be sterilized between use and commercially available chemical sterilization for human baby utensils works well
- do not enlarge the nipple hole as, whilst this makes feeding faster, pups swallow a lot of air and their stomachs become distended; worse still the pups can aspirate milk and this results in development of bronchopneumonia

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- it is easiest to hold the pup on your lap and hold its head elevated to allow it to suck from the bottle; the pup will normally paddle its front feet as a normal response during sucking
- it can be difficult to be sure of the exact volume of food taken by each pup

Tube feeding

- feeding by orogastric tube is quick, effective and allows the exact amount of food administered to be recorded
- normally a 6 French gauge soft plastic feeding tube with a rounded end is used
- the tube is placed alongside the pup and the distance between the mouth and the last rib is marked on the tube; ¾ of this distance is the length that will be placed into the pup
- the pup is best restrained on your lap and the tube is pushed gently into the pup's mouth; most pups will swallow the tube and it can be seen running down the left side of the neck (in the oesophagus)
- when neonates are being reared artificially, urination and defecation must be initiated after every meal by stimulating of the perineal region with cotton wool (Figure 15.3)
- the urine produced by well hydrated puppies should be colourless

Starting weaning

- solid foods can be introduced starting at 3 weeks of age by mixing puppy food with the milk formula and allowing it to soften
- this 'gruel' can be offered in small amounts four times a day; in many cases it is easiest to feed from a finger to get the pup's interest and then to place the finger into the gruel



Figure 15.3 In pups that are being artificially reared the perineal region needs to be stimulated to initiate urination and defecation.

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- as the pup gets older less milk can be used so the gruel is stiffened into a ‘paste’; usually no milk substitute is used at about 6 weeks of age
- as the dam is gradually removed from the pups they will start to play and rest together and will not be stressed by her absence; some pups settle better if they have an upended cardboard box in which to sleep
- pups should be encourage to establish a toileting area away from the sleeping area; here the paper can be replaced regularly
- all pups should be regularly handled, including opening their mouths, looking in their ears and exposing them to different sounds (see 1.1)

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Section 2

Abnormalities of Reproduction and the Perinatal Period

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16 Abnormalities of the Female

16.1 VULVA

Stenosis of the vulval opening

- this is rare condition where the vulval opening is too small
- this should be identified as part of a normal pre-breeding examination and, if not, it is noticed when the male is unable to penetrate at coitus and there is often coital pain affecting the female and sometimes the male
- surgical correction is not difficult and involves enlargement and suturing the mucosa to skin to prevent re-sealing

Perivulval skin fold dermatitis (Figure 16.1)

- this is relatively common in entire bitches and those that have been neutered either before or after the first oestrus
- sometimes incorrectly referred to as 'juvenile vulva' or 'vulval hypoplasia'; close inspection shows the vulva to be of normal size, but is either partially of completely hidden by folds of adjacent skin
- a large fold of skin dorsal to the vulva is responsible for the problem; often this is a crescent-shaped fold arising dorsal and lateral to the vulva
- an exudative dermatitis may develop in the skin folds surrounding the vulva; this is exacerbated by the bitch licking the area and by urine scalding
- in some cases the inflammatory response is severe resulting in pain which may make examination very difficult in the conscious animal because of the discomfort it causes
- conservative treatment consists of parenteral corticosteroids and antibiotics or locally applied anti-inflammatory creams; application of the latter is usually not well tolerated
- permanent treatments involve surgical ablation of the diseased skin and vulval lips with eradication of the sulci around the vulva

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Figure 16.1 Perivulval dermatitis. Severe inflammation of the vulva and perivulval skin fold.

Transmissible venereal tumour (Figure 16.2)

- present throughout the world with the exception of some island countries
- the tumour is caused by seeding of neoplastic cells which are transmitted during coitus
- verucose tumours arise in and around the genital tract
- not uncommonly there is seeding into the nasal cavity as the bitch licks the tumours
- they may be removed surgically or the bitch can be treated with a cytotoxic agent, e.g. vincristine



Figure 16.2 Transmissible venereal tumour affecting the vestibule-vulva.

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16.2 VESTIBULE

Congenital constrictions

- these can occur just inside the vulval lips
- usually digital examination reveals this fibrous bands running within the vulval lips
- in some cases there is trapping of material within the vestibule and vagina and a secondary bacterial overgrowth
- examination may be resented by the bitch but under general anaesthesia these constrictions can usually be dilated manually; recurrence is rare and there is not normally any obstruction at the time of parturition

Enlargement of the clitoris

- this may occur either congenitally (see intersexuality) or due to administration of androgens (see 18.4 and 27.3)
- frequently the clitoris develops a bone running along its long axis (os clitoris) similar to the os penis noted in the male
- the presence of an os clitoris frequently causes pain when the bitch sits down, or causes a vestibulitis resulting in excessive licking
- in some cases, if the problem is managed (e.g. by gonadectomy), as the bitch grows the relative size of the clitoris decreases and the clinical signs disappear
- in other cases it is necessary to undertake a clitoridectomy; in such cases it is necessary to establish to route of the urethra which may rarely run within the clitoris

Lymphoid hyperplasia (small nodules)

- small raised usually white nodules may be present within the vestibular or vaginal mucosa
- these nodules are sometimes traumatized at mating and may then bleed
- the nodules are probably a result of increased bacterial load for a variety of different reasons and are more commonly seen when there is intercurrent disease that allows bacterial overgrowth (e.g. vestibular constrictions)
- it has been suggested that these lesions are caused by an unknown virus, but this does not appear to be likely
- the lesions do not affect fertility (see 17.2)
- the appearance of the nodules can be easily differentiated from the vesicles seen with canine herpes virus infection

Canine herpes virus vesicles (see 21.5)

- some bitches exposed to canine herpes virus demonstrate vesicular lesions (often with submucosal haemorrhage) within the vagina and vestibule

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- these vesicles are initially fluid-filled (and are not solid like the pale-coloured lymphoid hyperplasia) before becoming eroded and leaving ulcer-like lesions
- viral recrudescence is a concern since lesions occur during oestrus and pregnancy, and may infect the male at the time of mating and puppies at parturition
- overall, however, mating is not the most common route of infection which is via the respiratory route

16.3 VAGINA

Strictures/stenosis

- abnormal development of the vagina is not uncommon at the junction of the vestibule and vagina
- most commonly lesions are found just cranial to the urethral opening
- the most common lesion appears to be a dorso-ventral vaginal band; if this is thin it may be broken easily but in some cases the band of tissue is very thick requiring an episiotomy approach or may not be amenable to surgical correction
- less commonly simple thin membrane constrictions which appear to be remnants of the hymen are found and these can easily be broken under general anaesthesia
- some cases have substantial narrowing of the vestibulo-vaginal junction associated with circumferential fibrous bands; these are more resistant to stretching and may continue forward some length along the vagina; these may be difficult to correct surgically, even via an episiotomy
- five aetiologies have been described:
  (a) the result of hypoplasia of the genital canal which causes vestibulo-vaginal hypoplasia and a vaginal stricture of some length
  (b) the result of poor or inadequate fusion of the Mullerian ducts to the urogenital sinus causing annular fibrous strictures at this site
  (c) tissue vestiges remaining at the vestibulo-vaginal junction resulting in hymenal remnants or a complete hymen
  (d) incomplete fusion of the two Mullerian ducts which may result in a double vagina or a vertical fibrous division within the vagina
  (e) imperfect joining of the genital folds and genital swellings resulting in vestibulo-vulval hypoplasia
- the extent and position of these lesions may be determined by contrast radiographic examination of the vagina

NB: Important research was conducted at the author's clinic which showed that bitches with severe abnormalities that could only be corrected by an episiotomy approach had lower pregnancy rate and greater whelping complications than bitches with mild abnormalities [1]; these data influence clinical

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practice since it cannot be recommended to treat and breed from bitches with severe abnormalities.

Hyperplasia of the vaginal mucosa (sometimes associated with protrusion) (Figure 16.3)

In some bitches with a normal endocrine environment there is substantial hyperplasia of the vaginal wall during pro-oestrus and oestrus.

- it is not clear why this occurs in some bitches and not others
- in some bitches it occurs at the first oestrus, in others at a later oestrus
- sequentially there appears to be a tongue-shaped thickening of the ventral vaginal wall just cranial to the external urethral orifice; this enlarges when oestrogen is elevated (pro-oestrus and oestrus) and regresses later in the luteal phase; sometimes the enlargement is so pronounced that there is protrusion of the mass of hyperplastic tissue
- some cases present because of pain at attempted coitus; others present because of the protrusion
- less commonly the entire circumference of the vaginal wall is affected resulting in a tubular mass of hyperplastic tissue that may also protrude
- most commonly the hyperplasia is seen to be progressively larger at each oestrus, although in some cases significant enlargement with protrusion is found at the pubertal oestrus



Figure 16.3 Vaginal hyperplasia and protrusion: (a) protrusion of the floor of the vagina; (b) protrusion of the whole circumference of the vagina.

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- the condition appears to be common in certain breeds (bullmastiffs) and certain lines within breeds
- when there is protrusion of the hyperplastic vagina the urethra is pulled caudally and the external urethral orifice (which is found on the ventral surface of the protrused tissue) ends outside of the vulval lips; this may cause urinary incontinence

Treatment

- conservative treatment of the hyperplastic and protruding tissue is needed to reduce trauma
- in the majority of cases this is followed by spontaneous resolution after the end of oestrus
- ovariectomy (ovariohysterectomy) will prevent recurrence of oestrus and therefore prevent the hyperplasia from developing again
- bitches intended for breeding find mating difficult – the dog tends to introduce his penis ventral to the tissue (Figure 16.3) and not into the vaginal cavity
- surgical removal may be considered and must be carried out during oestrus, when the full extent of the protrusion can be seen; visualization and control of haemorrhage are easiest if an episiotomy is performed first; care must be taken not to damage the urethra and this must be catheterized throughout the surgery
- the bitch can be mated at the oestrus following surgery; recurrence of the condition is uncommon after surgical excision

Vaginal tumours

- these are seen almost exclusively in entire bitches
- most cases are benign (fibromata, fibroleiomata, etc) and are often called polyps because they are pedunculated
- presenting signs are appearance of the polyp at the vulval lips, or haemorrhage due to ulceration of the surface
- surgical removal is usually feasible but may require episiotomy
- occasionally malignant tumours occur under the vaginal epithelium; they most commonly originate in the urethra

Vaginitis (see 5.5)

Definitions

- distinguishing between normal and abnormal vulval discharge can be difficult; a mucous and slightly purulent discharge is common at many stages of the cycle

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- identifying the source of vulval discharge can be difficult and the urinary tract should always be examined in any investigation
- defining vaginitis in the bitch is almost impossible because all bitches have a normal bacterial flora and have neutrophils in the vaginal smear (except during oestrus)
- a reasonable definition would be persistence of a copious purulent malodourous vulval discharge which may be associated with inflammation of the vaginal wall and irritation; some cases will also develop a secondary lymphoid hyperplasia

Differential diagnosis of vulval discharge

- Normal: pro-oestrus, oestrus, metoestrus, pregnancy, parturition and post-partum
- Abnormal: juvenile vaginitis, transmissible venereal tumour, ulcerated polyps, urethral tumours, vaginal trauma, vaginal foreign body, cystitis, pyometra, fetal death, placental separation, post-partum metritis and subinvolution of placental sites
- Urinary: ectopic ureter, post-spay incontinence, cystitis, urethral tumours

Diagnosis of vulval discharge

- in many cases the cause is obvious
- vaginal smears will distinguish pro-oestrus and oestrus from other causes (5.5)
- bacteriological swabs are of limited value because vaginal bacteria are common and normal (5.5); isolation of a pure growth of a single organism appears to be a more significant finding than a mixed growth, however primary bacterial vaginitis is very rare (except in countries where Brucella canis is present)
- in most cases there is a primary cause which is not bacterial and it is important to try to indentify this:
  (a) vaginoscopic examination is the most useful tool as it enables direct visualization of the reproductive tract and urinary tracts
  (b) radiographic contrast studies may reveal constrictions, tumours, foreign bodies and ectopic ureters

Pre-pubertal or juvenile vaginitis

- probably the most common cause of vaginitis is seen in the pre-pubertal bitch
- such cases may occur as early as 8 weeks of age when a copious creamy discharge develops
- there are no generalized signs of malaise
- may contain the normal bacterial flora or very few bacteria
- often more epithelial cells than neutrophils in the smear
- it is assumed that these cases have poor mucosal immunity which allows the overgrowth of commensal bacteria

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- generally there is only a transient improvement to systemic or topical antibiotics
- most cases resolve after the first oestrus (presumably because of oestrogen priming)
- in cases that are difficult to manage small doses of oestrogen may be administered; topical oestrogen creams are very useful in these cases, failing a response systemic oestogens may be administered

'Atrophic' vaginitis

- in some older bitches that have been neutered for many years a clinical syndrome similar to juvenile vaginitis develops
- in these cases no underlying cause is identified following standard work-up
- it is assumed that the aetiology is similar; poor mucosal immunity as a result of an absence of oestrogen
- these cases can be managed by topical or systemic oestrogens

Vaginal cysts

- these rare congenital abnormalities result from incomplete development of the para-mesonephric or Mullerian ducts
- they cause a substantial mass effect within the vagina which may interfere with breeding
- surgical removal is possible

16.4 CERVIX

- lesions of the cervix appear to be rare
- tumours occasionally occur but are usually asymptomatic
- rare cases of hyperplasia are seen; these may obstruct the cervical lumen and result in fluid pooling within the uterus (mucometra)
- any cervical abnormality may obstruct uterine fluid drainage

16.5 UTERUS

Aplasia

- congenital absence of the uterus is uncommon and when this occurs it is not uncommon for only a portion of the uterus to be affected
- if there is normal uterus proximal to an aplastic portion, secretions can accumulate and cause considerable enlargement of the uterus, which is usually asymptomatic (because there is no bacterial involvement)
- aplasia of one entire horn is called uterus unicornis

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- aplasia will cause reduced fertility since oocytes and sperm cannot meet on the affected side
- aplasia will result in sterility if it affects the uterine body, or both horns
- where there is suspicion of aplasia either laparoscopic examination or hysterography performed during oestrus can be diagnostic; some veterinary surgeons will resort to laparotomy (it is not always possible to image along the whole length of the normal uterine horn with ultrasound and so this has limited diagnostic ability)

Cystic endometrial hyperplasia

- during metoestrus (dioestrus) progesterone causes a thickening of the endometrium and massive development of the endometrial glands (see 2.6)
- normal size and appearance of the uterus when examined with ultrasound has been described from work conducted in the author's clinic [2]
- during anoestrus both these changes subside and there is some desquamation of epithelial tissue; however, the endometrium remains thicker than it was before puberty
- with succeeding cycles there is progressive permanent thickening of the endometrium and uterine glands become distended (cystic)
- early cases of cystic endometrial hyperplasia cause no clinical signs; grossly the uterus is thickened (1 cm or more in diameter) and appears to be twisted (Figure 16.4a)
- at this early stage cystic endometrial hyperplasia may, however, be associated with infertility
- the condition can be diagnosed using real-time ultrasound examination during the luteal phase (Figure 16.4b)
- bitches with cystic endometrial hyperplasia may have delayed clearance of uterine fluid after mating [3], and there is anecdotal evidence suggesting that

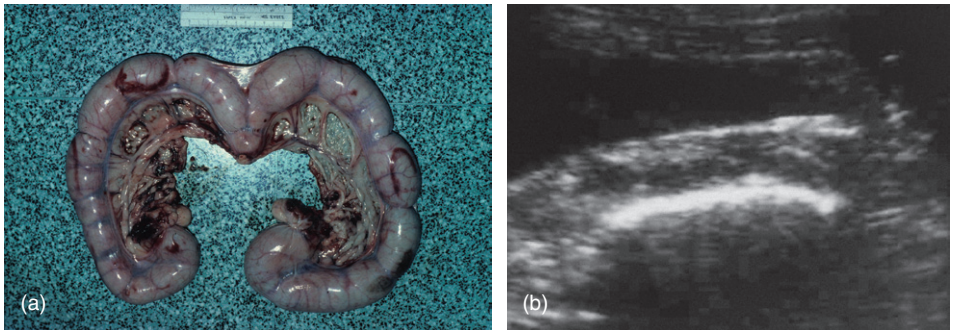


Figure 16.4 (a) Cystic endometrial hyperplasia; the uterus appears grossly twisted, but there is no exudate in the lumen and no inflammatory reaction. (b) Ultrasound image of the uterine body adjacent to the bladder containing distinct fluid-filled cystic regions. 5.0 MHz transducer, scale in centimetres.

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a short course of systemic broad-spectrum antibiotic immediately after mating [3] may improve pregnancy rates and litter size (presumably helping resolve bacterial contamination that occurs as a result of the retained fluid)
- it seems likely that administration of progesterone receptor antagonists to block the action of progesterone during one luteal phase may allow some resolution of the pathology which may increase fertility at the subsequent cycle, although this contention has yet to be proven

Endometritis

- there is no convincing evidence that 'low-grade' endometritis causes clinical signs in the bitch
- however in our clinic we have shown that bitches with cystic endometritis have greater numbers of luminal polymorphonuclear neutrophils after mating that do normal bitches [4]; and furthermore that this is associated with lower pregnancy rate and litter size
- in our studies we demonstrated that a 4-day course of broad-spectrum systemic antibiotic (for example potentiated sulphonamide) commencing immediately after mating could improve pregnancy rate and litter size in these bitches
- this suggests that bacterial contamination of the hyperplastic uterus may occur at oestrus

Pyometra (Figure 16.5) [5]

Aetiology

- the vagina has a normal resident bacterial flora (see 5.10)
- during oestrus the cervix relaxes and these bacteria are able to enter the uterus, especially during mating
- usually the defence mechanism of the uterus is competent at eliminating these bacteria, or some mechanism in the cervix (possibly mucus) may prevent their entry
- however, if bacteria do enter the uterus, the elevated blood progesterone concentrations at this time may reduce the speed of the inflammatory response (as in other species)
- in particular in bitches with cystic endometrial hyperplasia (i.e. older bitches) there seems to be reduced ability to eliminate these bacteria which may survive in the cyst fluid
- the growth of bacteria in the uterus eventually stimulates accumulation of inflammatory exudate, including large numbers of polymorphonuclear neutrophils
- the organism usually isolated from these cases is Escherichia coli, some strains of which may be more pathogenic than others; streptococci and other bacteria may also be present

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Figure 16.5 (a) Pyometra causing uniform enlargement of the whole uterus. (b) Pyometra causing partial dilation of the uterus. (c) Lateral radiograph of a bitch with pyometra; note that the enlarged uterus has displaced the colon dorsally and the small intestine cranially. (d) Ultrasound image of a uterus distended with pus. Three sections of the uterine horn are visible containing fluid with small echogenic particles. 5.0 MHz transducer, scale in centimetres.

- pathogencity appears to be related to the adherent ability of bacteria and commonly the uropathogenic organisms are isolated
- some cases of pyometra are said to be sterile; this may be related to the time of investigation, or because the samples collected were incorrectly handled before culture
- accumulation of pus in the uterus is eventually followed by the absorption of bacterial toxins into the circulation and signs of toxaemia
- in the author's laboratory we have documented changes in oestrogen and progesterone receptors in the uterus of bitches with pyometra compared with normal bitches, and it is likely that this is involved in the aetiology of the condition [6]
- there is no relationship between irregular oestrous cycles and the development of pyometra
- there is no relationship between pyometra and pseudopregnancy, although both may occur at the same time
- whether or not bitches which have never had a litter are more likely to develop pyometra is not known

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- the administration of synthetic progestogens may, as does endogenous progesterone, predispose to pyometra by causing cystic endometrial hyperplasia and by directly decreasing the resistance of the endometrium to bacterial invasion (see 27.1)
- administration of oestrogens to prevent conception (see 27.2) may predispose to pyometra by keeping the cervix relaxed for longer into the luteal phase than would normally be the case, and allowing bacterial colonization of the uterus
- an additional mechanism caused by local irritation by bacteria causing a trophoblastic reaction has been proposed
- in our laboratory we have shown that irritation of the endometrium can change the expression of progesterone receptors and this may therefore have some impact on the development of the condition [7]

Clinical signs

- in some cases the changes are not severe and the bitch has signs of malaise after oestrus but recovers at the end of the luteal phase; these bitches usually develop classical signs of pyometra at the subsequent cycle or the one after that
- initially there may be slight pyrexia and depression, but since signs of malaise are vague they are often not noticed
- sometimes the appearance of a purulent discharge at the vulva may precede signs of toxaemia, but usually the reverse is true
- toxaemia causes varying degrees of depression, anorexia, vomiting, polydipsia, polyuria and occasionally posterior paresis; when presented the body temperature may be normal or subnormal
- suppression of erythropoiesis may also occur due to toxaemia
- some cases of pyometra are closed, i.e. presumably the cervix is closed and does not allow the escape of pus, these bitches are often most severely ill
- in many cases, however, the cervix does open and there is release of exudate
- a classical discharge is purulent but may be yellow coloured or red–brown in nature
- despite the vulval discharge the majority of the pus is not expelled and is retained in the uterus
- the relationship between whether pyometra is ‘open’ or ‘closed’ and the location of pus in the uterus is unclear; in some bitches only one horn or part of one horn may be distended, in others the whole uterus is equally involved, and in a few there are discrete circular distensions separated by constrictions of the uterus, as in mid pregnancy
- in some bitches the distended uterus causes obvious abdominal enlargement

Diagnosis

- purulent vaginal discharge accompanied by clinical signs of malaise 4–6 weeks after oestrus is suggestive; however, the signs of toxaemia may develop before or after this

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- haematology will reveal a neutrophilia (usually in excess of 20×10^7/litre) with a 'shift to the left'
- radiography may identify a distended uterus causing dorsal displacement of the colon and cranial displacement of the small intestine; this must be distinguished from pregnancy (see 13.6)
- real-time ultrasonography will usually reveal a fluid-filled tubular viscus, devoid of fetal elements; the cranial uterine horn may also be imaged above the caudal part [8]
- palpation of the abdomen may detect uterine enlargement
- mammary development does not preclude the possibility of pyometra because this condition and pseudopregnancy are likely to occur at the same time
- laboratory tests may exclude other causes of polydipsia/polyuria, vomiting and paresis

Surgical treatment

- ovariohysterectomy is the treatment of choice if the bitch has a reasonable chance of survival; fluid therapy to rehydrate and antibiotics to prevent peritonitis are not optional

Medical treatment

- medical treatment of pyometra is possible, however in many cases there is recurrence at the next oestrus
- a reasonable prognostic indicator for recurrence is the thickness of the uterine wall which can be measured with ultrasound; when the uterine wall is greater than 5 mm in thickness and especially if endometrial cysts are present there is a high likelihood of recurrence, compared with bitches with a thin endometrium upon ultrasound examination
- if medical treatment is successful, it is likely that the condition will recur following the next oestrus; it may therefore be prudent to plan to breed the bitch at the first oestrus after treatment

Prolactin inhibitors combined with prostaglandins (see 27.9)

- daily oral administration of prolactin inhibitors (e.g. cabergoline at 5.0 µg/kg administered for 10 days) will remove support for the corpora lutea and result in a decrease in progesterone concentrations; as a result there will be softening and opening of the cervix, increased uterine contractions, and reduced endometrial secretion
- in our clinic we have shown that repeated administration of low-dose prostaglandin (e.g. cloprostenol at 5.0 µg/kg administered on day 3, 6 and 9) during cabergoline treatment caused active uterine contractions and good clinical efficacy [9]

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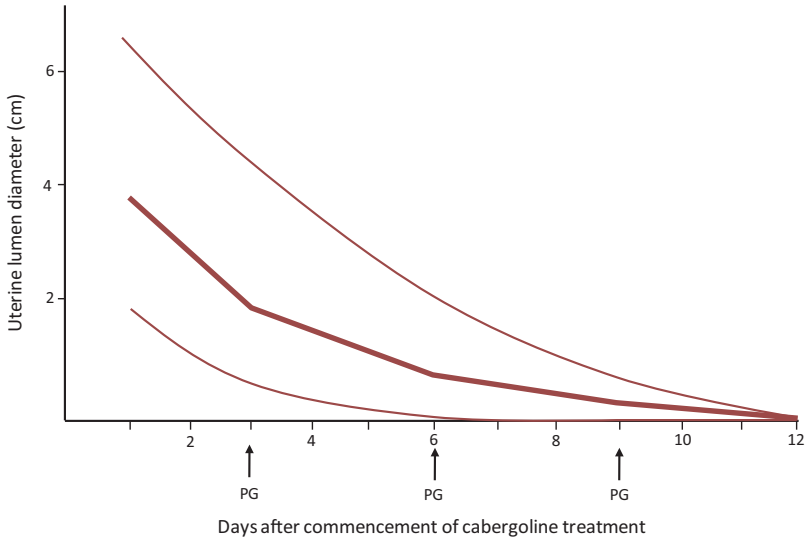


Figure 16.6 Reduction in uterine fluid depth (mean, max, min) in bitches with pyometra treated with daily cabergoline with prostaglandin (PG) administered on days 3, 6 and 9.

- such low doses of prostaglandin produce only short duration and mild adverse effects (salivation, vomiting, diarrhoea, dyspnoea, abdominal pain and tachycardia) but should be used with care
- broad-spectrum antimicrobial therapy should always be administered because 10% of bitches with pyometra are bacteraemic
- success rates are high at least for an immediate cure of the problem; on day 10 an ultrasound examination should be performed to ensure that all fluid has been expelled (Figure 16.6); if fluid remains present the treatment duration should be lengthened
- fertility rates at the subsequent oestrus are related to the degree of endometrial abnormality (thickness) which can be assessed using ultrasound examination; 30% of cases recur after the next oestrus

NB: This regime has replaced the use of prostaglandin alone; in particular prostaglandin alone should not be used in cases of closed-cervix pyometra because of the risk of uterine rupture.

Progesterone receptor antagonists (see 27.8)

- a small number of studies report successful treatment of pyometra using aglepristone (at 10 mg/kg on days 1, 3, 5, 8 and 16 after presentation)

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- antagonizing the progesterone will result in removal of its effect and the cervix will soften, there will be increased spontaneous uterine contractions and reduced endometrial secretion
- in clinical studies the regime produced no adverse effects, and emptying of uterine fluid occurred quickly, producing resolution of the clinical signs
- some of the bitches returned to normal fertility after treatment

NB: It would seem likely that in the future increased efficacy might be achieved using aglepristone combined with cloprostenol or cagerboline and cloprostenol.

Haemorrhagic discharge after oestrus

Two syndromes appear to be seen.

Delayed drainage of uterine fluid

- in bitches with vaginal hyperplasia or abnormalities at the vestibule-vaginal junction there may be trapping of uterine haemorrhagic discharge
- this fluid becomes changed in nature and develops a brown colour; it is slowly discharged over 10 days after oestrus, with the discharge decreasing each day
- no treatment is needed

Metorrhagia

- post-oestrus bleeding is seen in some older bitches
- the discharge is dark and unlike that in normal oestrus but continues to be large in volume
- there are no signs of malaise attributable to the condition
- a diagnosis of pyometra is often made, although this is erroneous
- ovariohysterectomy is, however, the only treatment
- the removed uterus is slightly enlarged and the ovaries contain 'cystic' structures; these may be adenomata although the aetiology of the condition requires further investigation

Uterine tumours

- uterine tumours are uncommon
- leiomyomata are seen in older bitches
- they are rarely associated with clinical signs which if they occur are usually a haemorrhagic discharge noted at non-oestrus phases of the cycle
- ultrasound examination is usually diagnostic demonstrating focal uterine enlargement and trapped fluid

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16.6 UTERINE TUBES

- there is very little information relating to uterine tube abnormalities in the bitch
- a small number of cases of uterine tube inflammation have been demonstrated, but this does not appear to be common and tubal obstruction appears to be sufficiently rare to not be considered a differential diagnosis for infertility
- occasional tumours occur

16.7 OVARIES

Ovarian cysts

- true ovarian cysts are very rare, and many cases that are 'diagnosed' at the time of ovariohysterectomy are in fact cystic structures within the ovarian bursa. Both follicular and luteal cysts may be seen

Follicular cysts

- cystic follicles are frequently over-diagnosed based on clinical signs; most bitches with prolonged signs of pro-oestrus or oestrus are normal and do not have ovarian cysts
- true cystic follicles do result in persistent oestrus but a duration of more than 30 days should elapse before diagnostic work-up commences since normal pro-oestrus and oestrus may last 30 days
- ultrasound examination at this time will demonstrate multiple thin-walled fluid-filled (anechoic) structures often 10–12 mm in internal diameter
- follicular cysts may be forced to ovulate by the administration of exogenous human chorionic gonadotrophin (hCG) which is LH-like in activity; often daily or alternate day administration is needed over 3–6 days
- in cases that do not respond to hCG it may be possible to cause regression of the cyst using oral progestogens (e.g. megoestrol acetate) although there is a risk of pyometra since progesterone is being administered at at time when endogenous oestrogen concentrations are high

Luteal cysts

- luteal cysts are rare and whilst progesterone is produced the most common clinical sign is absence of oestrus
- in some cases luteal cysts are identified in bitches with pyometra; it seems likely that a persistent elevation of progesterone predisposes to pyometra, but this absolute relationship is unproven
- luteal cysts can be diagnosed by elevated progesterone and presence of single or multiple thick-walled fluid-filled structures within the ovaries

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- some anecdotal reports suggest that luteal cysts may respond to prostaglandin administration, however there is inadequate evidence for this contention

Ovarian tumours

- ovarian tumours are most often diagnosed either because of the endocrine consequences (production of oestrogen and vague signs of oestrus that wax and wane) or because of a mass effect and ascites
- granulosa cell tumours are usually associated with persistent or recurrent oestrus; both plasma oestrogens and progesterone concentrations are reported to be elevated
- dysgerminomata and teratomata are endocrinologically inactive but may be suspected if abdominal distention and ascites are present
- adenomata (cyst adenomata) may affect both ovaries but are normally benign and associated with no clinical signs (their possible association with metorrhagia (see 16.5) is unclear)
- diagnosis of ovarian tumours may be made on the basis of clinical signs, palpation, radiography, ultrasonography and plasma steroid assay
- surgical removal is usually effective unless metastases have occurred
- care should be taken not to rupture these neoplasms since metastatic spread is often by trans-coelomic seeding

16.8 MAMMARY GLAND

Asymetrical mammary glands

- this is common and a normal anatomical variant

Accessory nipples

- these small additional nipples are found on normal mammary glands and may be functional and connect to the same collecting ducts as the main nipple; milk will be emitted in the lactating bitch
- most cases need no treatment

Inverted nipples

- in some bitches nipples are inverted and trap waxy material
- these may require chronic management care including lubricants and topical antibiotics; in other cases the nipple everts and does not cause a problem during lactation

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Hyperplasia of nipples

- a hyperplastic response of the nipple epithelium can be found following chronic inflammation associated either with damage during sucking or due to contact with an irritant cleaning fluid or bedding
- most cases require no treatment but are unsightly; rarely they interfere with sucking because the teat is too large for the pup's mouth

Fibroadenomatous mammary hyperplasia

- hyperplasia of one or two of the caudal mammary glands is occasionally seen in bitches
- the condition is recognised by a multi-nodular (5–10 mm diameter) fluid-filled firm to almost spongy enlargement of the glands
- the gland is non-painful but a brown discharge may be present
- it is most commonly seen during the luteal phase and regresses significantly at the end of the luteal phase
- in the acute situation termination of the luteal phase using prolactin inhibitors, or blocking the action of progesterone using a progesterone antagonist can produce a clinical response
- surgical neutering prevents recurrence
- in bitches required for breeding it may be necessary to remove the affected gland(s)

Mammary gland neoplasia

- mammary tumours are common in bitches but are infrequently seen in bitches that are neutered before puberty or in the first year of life
- canine mammary tumours are carcinoma (with six types and additional sub-types), sarcoma (four types), carcinosarcoma (mixed mammary tumours), or benign adenoma
- obesity appears to be a risk factor for development of mammary neoplasia
- 50% of tumours are malignant and bitches therefore require appropriate screening (e.g. thoracic radiography, blood biochemistry)
- clinical features that increase the suspicion of malignancy include:
  (a) rapid growth of the mass
  (b) poor zone of transition to the adjacent tissue
  (c) a tumour larger than 2.5 cm in diameter
  (d) enlargement of the local lymph node
  (e) oedema of local tissue
- the prognosis for most mammary tumours whether benign or malignant is favourable as long as they receive rapid surgical treatment

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- clinical staging follows the World Health Organization criteria:

Description	Classification
Tumour less than 3 cm diameter	T1
Tumour 3–5 cm diameter	T2
Tumour over 5 cm in diameter	T3
No metastasis to local node on histopathology	N0
Metastasis to local node identified on histopathology	N1
No distant metastasis identified	M0
Distant metastasis identified	M1

- stages are then defined as:

Classification summary	Stage
T1, N0, M0	I
T2, N0, M0	II
T3, N0, M0	III
any T, N1, M0	IV
any T, any N, M1	V

- small tumours can be removed by nodulectomy followed by histopathological examination
- masses that are 2 cm in size may warrant fine-needle aspiration before further surgical treatment; simple mastectomy will be indicated in well differentiated tumours whilst block, chain or total mastectomy may be required for poorly differentiated tumours
- recurrence for poorly differentiated canine mammary tumours is 90%; for moderately differentiated tumours, 68%; and for well differentiated tumours 24%

NB: The decision about which mammary gland to remove is dependent upon the lymphatic drainage from the mammary gland; the most cranial and caudal glands drain direct to the accessory and inguinal lymph nodes respectively (see 2.11) but other glands have lymphatic connections to adjacent glands.

As a general guide for surgery:

Tumour in gland 1	remove gland 1
Tumour gland 2	remove gland 2 and 1
Tumour in gland 3	remove all glands
Tumour in gland 4	remove gland 4 and 5
Tumour in gland 5	remove gland 5

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- Administration of a prolactin inhibitor before surgery can be useful since this reduces any mammary gland hyperplasia, improves visualization of tumour edges, reduces tumour size and overall makes the surgery easier
- Ovariohysterectomy or ovariectomy is recommended to prevent development of new neoplasia
- Triple chemotherapy treatment (cyclophosphamide, doxorubicin and fluorouracil) has been advocated when metastatic spread is confirmed or suspected

16.9 URETHRA

Urethral sphincter mechanism incompetence

- following surgical neutering up to 20% of bitches develop urinary incompetence caused by insufficient closure of the urethral sphincter; bitches show overflow incontinence often when recumbent
- some bitches develop signs within a few months of surgery, in most it is several years
- neutered bitches have low plasma concentrations of oestrogen and elevated concentrations of GnRH, LH and FSH
- medical treatments are aimed at improving sphincter closure either by administration of:
  (a) alpha-adrenergic drugs; drugs such as phenylpropanolamine act selectively on alpha-1 receptors, these are present in the bladder neck and cranial urethra, stimulation causes increased closure pressure. Approximately 75% of bitches respond to alpha-adrenergic drugs
  (b) oestogens; drugs such as oestriol sensitize the alpha receptors to other endogenous hormones and therefore increase urethral closure pressure. Approximately 65% of bitches respond to oestrogens, interestingly sometimes these are different bitches to those that respond to alpha-adrenergic drugs so if one regime fails it is worth trying another
  (c) GnRH agonists; depot agonists such as deslorelin implants cause a reduction in LH and FSH concentrations and in some bitches these produces a clinical response but the mechanism is not fully elucidated. Approximately 50% of bitches have some response to GnRH agonist implants
- surgical treatments are aimed at changing urethral position or anatomy so that closure pressure is increased, surgery is normally indicated in cases that are refractory to medical treatment:
  (a) colposuspension: the bladder neck and proximal urethra are relocated into the abdomen by drawing forward the cranial vaginal remnant and anchoring it to the prepubic tendon using nylon sutures
  (b) urethropexy: the urethra is relocated into the abdomen and tension on the urethra increases resistance to flow by drawing forward the urethra and anchoring it to the prepubic tendon using nylon sutures
  (c) urethral submucosal collagen: the urethral lumen is narrowed by endoscopic injection of collagen into the urethral submucosa

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Other urethral abnormalities

- these are not common
- most frequently seen is transitional cell carcinoma which may be local spread from the bladder neck; these may present as a potential reproductive tract problem because clinical signs are usually a persistent haemorrhagic vulval discharge. These cases require endoscopic examination or a positive contrast vagino-urethrogram where the neoplasm can usually be readily identified

REFERENCES

1 Moxon, R. and England, G.C.W. (2011) Fertility and whelping complications in bitches following vaginal abnormality repair. *Veterinary Record* **168**, 642–644.

2 England, G.C.W. and Yeager, A.E. (1993) Ultrasonographic appearance of the ovary and uterus of the bitch during oestrus, ovulation and early pregnancy. *Journal of Reproduction and Fertility* **47**(Supplement), 107–117.

3 England, G.C.W., Moxon, R. and Freeman, S.L. (2012) Delayed uterine fluid clearance and reduced uterine perfusion in bitches with endometrial hyperplasia and clinical management with post-mating antibiotic. *Therigenology* (in press).

4 England, G.C.W., Burgess, C.M. and Freeman, S.L. (2012) Perturbed sperm-epithelial interaction in bitches with mating-induced endometritis. *The Veterinary Journal* (in press).

5 Noakes, D.E.N., Dhaliwal, G. and England, G.C.W. (2001) Cystic endometrial hyperplasia/pyometra in the dog: a review. *Journal of Reproduction and Fertility* **57**(Supplement), 395–406.

6 Dhaliwal, G.K., England, G.C.W. and Noakes, D.E.N. (1999) Oestrogen and progesterone receptors in the uterine wall of bitches with clinical signs of cystic endometrial hyperplasia/pyometra. *Veterinary Record* **145**, 455–457.

7 Dhaliwal, G.K., England, G.C.W. and Noakes, D.E. (2002) The effects of endometrial scarification on uterine steroid receptors, bacterial flora and histological structure in the bitch. *Animal Reproduction Science* **15**, 239–249.

8 England, G.C.W. and Allen, W.E. (1990) Ultrasound diagnosis of pregnancy and pyometra in the bitch. *Veterinary Annual* **30**, 217–222.

9 England, G.C.W., Freeman, S.L. and Russo, M. (2007) Treatment of spontaneous pyometra in the bitch with a combination of cabergoline and prostaglandin. *Veterinary Record* **160**, 293–296.

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17 Abnormalities of the Male

17.1 PREPUCE (SHEATH)

Phimosis

This is constriction of the preputial orifice such that the penis cannot be exteriorized.

- it may occur congenitally or as the result of trauma or inflammation which results in a secondary fibrous change to the sheath
- in severe cases the preputial orifice is so small that the penile tip cannot be protruded and urine cannot escape as it is voided; it accumulates in the sheath and dribbles thereafter from the preputial opening
- these dogs often may either stand with urine dribbling or urinate on the caudal aspect of their fore legs
- affected dogs are not able to copulate and show signs of pain during erection
- surgical enlargement of the orifice is usually curative

Paraphimosis

This occurs when the penis can be exposed from the sheath, but cannot be retracted.

- it is not always an absolute disparity between the size of the preputial orifice and the size of the penis
- in some dogs after normal copulation the penis is slow to return to the sheath, or causes inversion of the preputial skin and hair; lubrication of the penis with a water-soluble gel will aid its return, although the dog may resent manipulation of the penis – failure to help causes paraphimosis
- spinal lesions may also be associated with paraphimosis
- foreign bodies may cause paraphimosis

Dog Breeding, Whelping and Puppy Care, First Edition. Gary C.W. England.
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- if the erect penis cannot be detumified, it soon becomes dry and cyanotic; the application of ice packs and lubricants, administration of sedatives or ultimately replacement under general anaesthesia may be required to reduce swelling
- subsequent surgical enlargement of the preputial orifice may prevent recurrence
- on occasions the exact cause of the condition is unclear and correction may ultimately necessitate amputation of the a portion of the penis

Priapism

This is persistent enlargement of the penis in the absence of sexual excitement.

- it may be the result of neurogenic abnormalities such as lumbar spinal lesions
- conservative management including repeated replacement of the penis in the sheath may be successful, although some cases require partial penile amputation

Short prepuce

In some dogs there is a relative size mismatch between the length of the penis and the length of the prepuce which results in the distal tip of the penis being exposed when not erect.

- in these dogs the penile tip may desiccate and there is frequently excessive licking and self-trauma
- some cases can be managed using protective creams but in others a permanent solution is required which may be either preputial advancement or partial penile amputation

NB: Where the abnormality is combined with incorrectly positioned orifices intersexuality should be considered (see 18.4).

Urination on the chest and front legs

- seen in some King Charles spaniels
- despite lifting a hind leg during urination, urine is deposited on the ventral thorax and the back of one or both front legs
- this causes problems for the owner who either has to wash the dog several times a day or 'put up with' the smell
- the cause is unknown
- surgery to pull the prepuce forward and thus deviate the urine ventrally is rarely curative

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- amputation of the distal penis allows the now empty prepuce to direct the urine away from the body

Balanitis

- this is inflammation of the lining of the sheath
- the condition is rare and is frequently an erroneous diagnosis (see balanoposthitis)

17.2 PENIS

Penile hypoplasia

- under-development of the penis is extremely rare but reported in certain breeds
- may be due to sex chromosome abnormalities
- the clinical signs are related to urine pooling within the prepuce

Hypospadias

- this is opening of the urethra somewhere along the ventral surface of the penis, and not at its tip; it may be as far back as the anus
- six classifications have been described, of which the perineal form is the most common
- invariably this condition occurs in intersex dogs (see 18.4)
- affected dogs may also have a short and deviated penis
- reconstructive surgery or penile amputation may be required

Persistent penile frenulum

- this is a band of tissue attaching the ventral surface of the penis to the adjacent prepuce
- in normal dogs the frenulum has usually disappeared by puberty
- if it persists, attempts to extrude the penis from the prepuce may cause pain, ventral deviation of the penis or haemorrhage if the frenulum ruptures
- surgical section of the frenulum under general anaesthesia may be necessary
- a heritable nature has been established in one breed

Haemorrhage

- haemorrhage through the penile integument may occur apparently spontaneously or after known trauma, e.g. dog bite

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- torn areas of integument rarely heal well and sometimes fistulae develop to the underlying cavernous tissue so that the lesions leak blood which can be at substantial pressure during erection
- mattress sutures of fine absorbable material should be used to occlude the lesion under general anaesthesia
- if lesions are refractory to this treatment, the penis should be amputated and the dog castrated (in order to try to avoid the dog obtaining an erection)

Fracture of the os penis

- traumatic injuries to the penis may result in the os becoming fractured
- usually there is significant urethral haemorrhage in the early phases and there may be immediate or later dysuria due to urethral obstruction
- diagnosis is by palpation and radiographic examination
- treatment initially is to allow healing to occur unaided
- indwelling urethral catheters may be useful during the initial healing phase
- if callus formation causes urethral constriction, penile amputation or perineal or scrotal urethrostomy should be considered

Lymphoid hyperplasia

- pale 1–2 mm diameter nodules seen at the base of the glans penis close to the reflection of the sheath, and in some cases lesions are also seen on the inner preputial lining
- the condition appears very similar to that noted in bitches
- the condition has wrongly been called ‘dog pox’; the lesions are, however, not vesicles, and on this basis and the pale colour can also be differentiated from canine herpes virus lesions
- lesions are often found incidentally during routine fertility examination often in dogs where there is a heavy preputial discharge; presumably they occur as a result of high commensal bacterial load within the prepuce
- occasionally these nodules may be abraded during mating or semen collection

Posthitis

- this is inflammation of the penis and appears as congestion-like discoloration of the penis
- it is seen in some dogs which masturbate, or occasionally when trauma occurs during mating or when semen collection is attempted by an inexperienced person
- conservative treatment is required in most cases

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Balanoposthitis

- inflammation of the penis and the inner preputial lining is very rare
- dogs with excessive purulent discharge from the prepuce are often said to have balanoposthitis (inflammation of the penis and sheath) but this is usually an erroneous diagnosis because:
  (a) all entire male dogs have some discharge from the prepuce
  (b) close inspection of the penis and sheath usually reveals no sign of inflammation
  (c) swabs and smears of the surface of the penis will reveal macrophages, polymorphonuclear leucocytes and bacteria, which are all normal
  (d) Ureoplasma and Mycoplasma are normal in the dog prepuce and semen

Preputial discharge

- as noted above all dogs have some preputial discharge which is normal; it may drip from the prepuce or be licked away by the dog (Figure 17.1)
- it is difficult to define excessive discharge; this may depend on the dog's owner, i.e. what is acceptable in a kennelled dog may not be acceptable in a dog which sits on furniture
- the cause of excessive discharge in dogs which do not have obvious lesions, e.g. foreign bodies is not known
- during routine fertility examinations, quite copious accumulations of pus may be seen with or without lymphoid hyperplasia and are considered normal
- bacteriological culture frequently reveals normal commensal organisms
- culture of an antibiotic-resistant bacterium, e.g. Pseudomonas aeruginosa, or any other organism in pure culture, may be significant, but this occurs very rarely

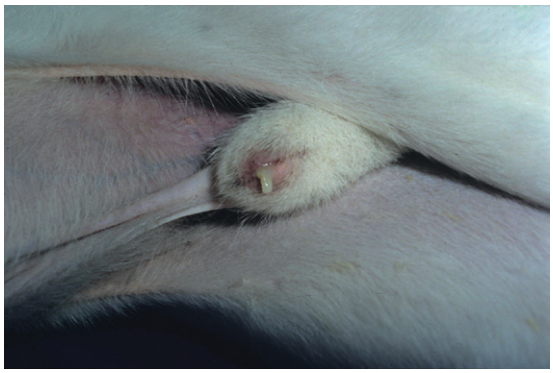


Figure 17.1 Photograph of the sheath of a dog showing the normal volume of preputial discharge which may drip from the prepuce or be licked away by the dog.

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- parenteral treatment with antibiotics is usually not necessary and is not effective
- parenteral treatment with sex steroids to reduce sexual activity and self-interest may help; if so, castration may be a permanent cure
- flushing the prepuce (general anaesthesia may be necessary) with saline followed by topical application of antibiotic creams may produce a temporary control of the clinical signs; some cases need to be treated regularly before the sheath is 're-seeded' with commensal bacterial collected from another dog
- prolonged topical antibiotic treatment alone should be avoided if possible since it may select for other resistant organisms

NB: Pus may originate from the prostate gland but the dog invariably shows other signs of prostate disease (see 17.7). Reduction in pus production by the normal prepuce/penis may be impossible because it is probably stimulated by as yet unidentified factors.

Penile neoplasms

- in countries free from transmissible venereal tumour, neoplasia of the penis is rare
- squamous cell carcinomas are the most usual type and commonly spread locally and metastasize to inguinal lymph nodes
- haemorrhage from an ulcerated neoplasm may be the first clinical sign
- in the absence of metastases radical surgical resection is warranted

Transmissible venereal tumour

- present in many countries especially southern Europe and Africa
- an interesting tumour biologically which is transmitted coitally by 'seeding' of infected cells
- lesions are usually friable, can be large and ulcerated and often haemorrhagic (Figure 17.2)
- the tumour is not normally found within the UK
- local excision, amputation and chemotherapy (vincristine) may be suitable treatments depending upon the location and extent of the tumour
- spread to the nose and nasal cavity is commonly seen as a result of dogs licking the primary lesion

17.3 TESTES

Ultrasound examination of the testes and epididymides should always be part of the clinical examination in any cases of testicular pathology; in our laboratory we have led the use of this technology [1,2,3] which has now a wide use in clinical practice.

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Figure 17.2 Photograph of the penis of a dog showing the presence of a tumour-like mass that was subsequently found to be a transmissible venereal tumour. Such lesions are usually friable, ulcerated and often haemorrhagic.

Anorchism

This is congenital absence of both testes.

- there is only one documented case of a male dog being born without testicles, reported by the author's clinic [4]
- in most cases the animal has two testes neither of which are within the scrotum and the term 'anorchid' is used inappropriately
- anorchidism and previous castration can be confirmed by a human chorionic gonadotrophin (hCG) stimulation test (see 10.15); failure of testosterone concentrations to increase after hCG administration is diagnostic

Monorchidism

This is the condition of a dog having only one testicle.

- it has not been reported as a congenital condition but can exist after surgical removal of one testis
- the condition is not common and most cases are in fact unilateral cryptorchid (i.e. they have one testis within the scrotum and one elsewhere)

Cryptorchidism

This is a condition in which there is failure of one or both testes to enter the scrotum.

- the testes may pass through the inguinal canal or not
- depending on whether both or one testicle fails to descend, the dog is called a bilateral or unilateral cryptorchid (not monorchid)

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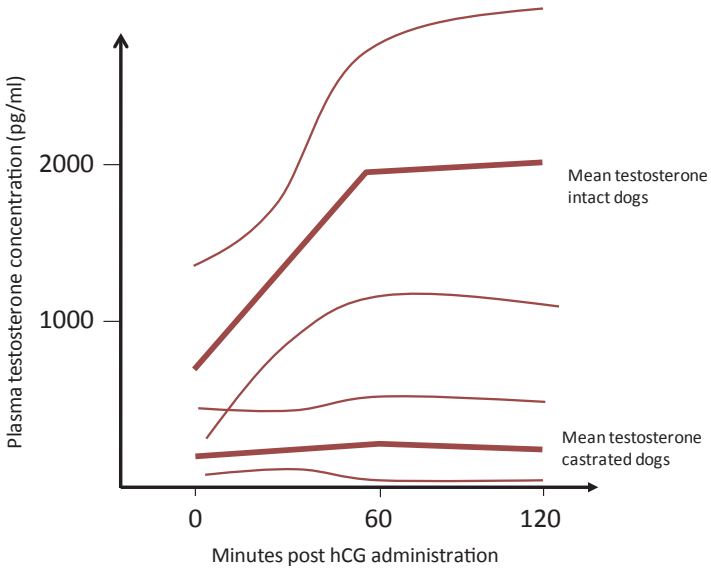


Figure 17.3 Changes in plasma testosterone (mean, max, min) before and after administration of hCG. NB: There is overlap of testosterone concentration in the intact and castrated dogs prior to hCG administration.

- testes which are not within the scrotum still produce testosterone but generally they do not produce spermatozoa because of the higher temperature; this means that unilateral cryptorchids are usually fertile but bilateral cryptorchids are not
- cryptorchid dogs usually have normal libido
- testes are normally descended by 10 days of age but may be difficult to palpate in young pups due to their small size and the presence of scrotal fat; therefore a diagnosis is usually not confirmed until 10 weeks of age
- bilateral cryptorchids can be diagnosed by a significant increase in testosterone following the administration of hCG (Figure 17.3); a diagnostic test validated by the author's laboratory [5]
- as cryptorchid dogs get older, the chance of normal descent becomes more remote; it is very rare after 1 year of age
- sometimes fully descended testes in pups appear to become abdominal again; the reason for this is not known
- there is no reliable medical treatment shown to be efficacious for inducing testicular descent
- in any event, medical treatment is not ethical since the condition has a genetic base
- the mode of inheritance is not fully known but it follows the model of a sex-limited autosomal recessive trait:
  (a) both female and male parents will carry the gene
  (b) homozygous males will be cryptorchid

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- treatment is removal of the abdominal testis surgically because these testes are more likely to become neoplastic than scrotal testes
- removal of the other testis at the same time is desirable to prevent the dog from breeding
- surgical removal of the abdominal testis is not necessary until 18 months of age, as neoplastic changes are unlikely before this time

Ectopic testes

These are testes which do not occupy a normal scrotal position; essentially these are cryptorchid dogs where a different terminology is being used; in the author's opinion the mode of inheritance is sex-limited autosomal recessive.

- the abdominal testes of cryptorchid dogs are therefore ectopic
- testes which have passed through the inguinal canal may then migrate away from the scrotum to become ectopic
- these testes may be found:
  (a) anterior to the scrotum, under the skin and lateral to the penis; these may be palpated under the skin
  (b) in the femoral triangle; these are not palpable
  (c) caudally in the perineum, lateral to the corpus cavernosum; these may be palpable
- treatment is as for cryptorchid dogs

Testicular hypoplasia

- scrotal testes which do not develop properly, i.e. remain small after birth, are rare; small testes are usually the result of degeneration (see Testicular degeneration)

Traumatic orchitis (inflammation of the testis)

- this may occur during any incident, but is commonly the result of dog fights
- any lesion to the scrotal skin results in excessive licking and secondary self-trauma
- conservative therapy includes local cleansing, antibiosis, diuretics to reduce swelling and, importantly, measures to prevent self-trauma
- deep testicular lesions rarely heal well and may necessitate castration
- healing is often followed by degeneration of the testis because of the increased temperature that was experienced
- not uncommonly the contralateral testis is also affected by testicular degeneration, emphasizing the need for rapid and aggressive treatment of any case of orchitis if fertility is to be salvaged

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Non-traumatic orchitis

- in some countries Brucella canis infection causes acute orchitis and epididymitis with sperm abnormalities and secondary infertililty
- the condition can be transmitted at coitus and screening programmes are needed for breeding dogs and bitches
- following Brucella infection the testes become indurated and testicular degeneration ensues
- treatment of Brucella canis is difficult (see 21.5)
- Brucella canis is not present in the UK, but it is possible that this condition will be introduced as a result of transportation of dogs to Europe for breeding
- in the absence of Brucella a bacterial inflammation of the testis via ascending infection or the haematogenous route is rare although in a small number of dogs a degenerative orchitis can be associated with autoimmune thyroiditis
- some dogs which show no clinical signs of disease of the testes or prostate gland produce ejaculates containing large numbers of inflammatory cells; their origin is unknown (the surface of the penis must be eliminated as the source)

Testicular torsion

- this is more correctly torsion of the spermatic cord
- the cause of the torsion is unknown but appears to be associated with recent exercise in many cases
- the condition is essentially a rotation of the testis within the scrotum
- as a result of the torsion the pampiniform plexus (see 6.6) becomes compressed but blood continues to enter the testis via the spermatic artery; this causes rapid swelling of the testis but since the testicular capsule is very fibrous the swelling is restricted resulting in marked pain such that the dog is reluctant to move
- after a few hours the testis may be significantly damaged and at this time palpation is no longer painful
- diagnosis is relatively simple because there is swelling of the pampiniform plexus, epididymis and testis (Figure 17.4)
- frequently there is self-trauma to the scrotum because of the pain
- haematuria may be a presenting sign
- diagnosis is easy when testes are present within the scrotum, however the condition may also occur in abdominal testes when there is acute abdominal pain; the cause of which may only be diagnosed at exploratory laparotomy
- surgical removal of the affected testis gives rapid pain relief and is always warranted as the testis will never function properly again
- unless the condition is rapidly treated surgically the contralateral testis will be affected by the increase in scrotal temperature and may suffer testicular degeneration

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Figure 17.4 Photograph of the scrotal contents of a dog following castration. In one there was torsion of the spermatic cord, resulting in swelling of the pampiniform plexus, epidiymis and testis. The contralateral testis is normal.

Testicular degeneration

- a variety of initiating insults have been proposed including: testicular heating (scrotal trauma, orchitis, testicular torsion), prolonged exposure to androgens (either exogenous or endogenous for example a hormonally active testicular neoplasm), some endocrinopathies, diabetes mellitus, presumed but unknown toxins, and in some breeds an immune-mediated problem (see spermatogenic arrest)
- early in the disease the testes may change in texture and semen quality may be affected
- in the mid-course of the disease the testes become soft and smaller than normal; ultrasonographically zones of increased echogenicity (probably representing calcification or fibrous tissue deposition) develop throughout the affected testis; at this time the dog may be almost azoospermic [1]
- late in the course of the condition the affected testes are small, hard and the surface may be uneven; at this stage an irreversible azoospermia is present
- usually both the spermatogenic and endocrine functions of the testis are lost:
  (a) plasma testosterone concentrations are low
  (b) plasma LH and FSH concentrations are high
- no treatments have been shown to be efficacious for established testicular degeneration

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Abnormal spermatogenesis [6]

- this is the cause of some abnormal ejaculates associated with infertility
- the affected testes may be normal, soft or small
- the condition may be permanent for unknown reasons, or temporary, as caused by pyrexia (see 6.6) and some drugs (see Chapter 27) [7]
- there may be increased or decreased echogenicity of the testicular parenchyma when examined with ultrasound
- diagnosis may, however, require fine-needle aspiration; testicular biopsy is not recommended since the sequelae of the procedure are severe
- there is no proven effective treatment although in a small number of dogs administration of the synthetic androgen mesterolone (an androgen that cannot be aromatized to oestrogen and therefore has minimal negative feedback effects upon the hypothalamus and pituitary gland) has been shown by the author to marginally increase semen quality [8]; this may offer the only potential treatment available in clinical practice

NB: The author has found no evidence that vaccination against parvovirus disease causes infertility in dogs [9] nor that specific antibiotics have adverse effects on semen quality [10].

Spermatogenic arrest (see 10.5)

- this is a specific testicular degeneration
- affected dogs are usually fertile initially
- spermatogenesis stops suddenly, often between 18 months and 7 years of age
- semen collection shows the presence of prostatic fluid and no spermatozoa
- the dog shows no signs of malaise
- the testes may be smaller and softer than normal
- the condition is irreversible
- it is probably an autoimmune disease
- it is inherited in some breeds including the Welsh springer spaniel and the Labrador retriever
- diagnosis can be made on the basis of semen evaluation, changes in testicular ultrasonographic appearance, and fine-needle aspiration
- data from the author's clinic shows that typical ultrasound changes include increased parenchymal echogenicity, echogenic stippling and reduced testicular volume [1]; these findings have enhanced our ability to diagnose these cases in clinical practice
- early in the disease process many abnormal spermatozoa may be found within the ejaculate
- there is no proven effective treatment; the synthetic androgen, mesterolone, does not appear to prevent progression of the condition

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Relationship of semen quality to fertility

Normal semen quality

- on average semen quality is superior in fertile compared with non-fertile samples
- however, there is no unimpeachable method of predicting the fertility of a semen sample
- 'normal values' from a variety of breeds are given in Table 17.1
- some dogs with poor semen quality may be fertile using assisted reproductive techniques
- indeed the author's laboratory documented the first data on semen quality from fertile males and showed a surprising range of qualities that were fertile [11]

Table 17.1 Characteristics of the second fraction of the ejaculate from 53 fertile dogs.

	Motility (%)	Volume (ml)	Concentration ($\times 10^6$)/ml	Total sperm output ($\times 10^6$)	Live normal (%)	Dead normal (%)	Primary abnormal (%)
Mean	85.2	1.3	310.5	403.4	75.2	10.2	1.6
S.D.	6.2	0.4	82	120	7.9	5.4	2.6
Range	42–92	0.4–3.4	50–560	36–620	52–90	2–26	0.1–2.0

Spermatozoal number

- an inverse relationship has been established between ejaculate volume and spermatozoal concentration
- there are also differences between large and small breeds of dog
- the determination of the total number of spermatozoa per ejaculate is the most accurate measure of sperm production
- infertility in the dog has not been associated simply with reduced spermatozoal numbers, although irreversible azoospermia in previously fertile dogs has been recognized for some time
- cases of functional azoospermia with spermatogenic arrest have been recorded in two related Welsh springer spaniels and in some related Labrador retrievers
- one case of azoospermia has been reported which was reversed following sexual rest
- a seasonal effect on spermatozoal production has been noted in dogs in warm climates

Spermatozoal morphology

- a high incidence of morphologically abnormal spermatozoa is often associated with reduced fertility

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- there have been few reports concerning the influence of specific morphological abnormalities on fertility
- abnormal mid piece droplets were identified in an infertile greyhound dog
- abnormal head-base/mid piece regions have also been associated with infertility:
  (a) the commonest abnormality was swelling of the mid piece region
  (b) electron microscopic studies showed axonemal and mitochondrial defects both within these membrane swellings and at other regions of the mid piece
- spermatozoa with mid piece defects and deformed acrosomes have been found associated with experimental Brucella canis infection
- no other spermatozoal morphological abnormalities have been reported to affect fertility in the dog
- however, the relationship between the percentage of morphologically abnormal spermatozoa and fertility of the dog has been established:
  (a) the fertility of dogs with greater than 60% morphologically normal spermatozoa was 61%
  (b) the fertility for those with less than 60% morphologically normal spermatozoa was 13%

Live:dead (vital) staining

- staining of spermatozoa with a vital stain is considered part of the normal morphological assessment
- in several species there have been good correlations found between the percentage of vital staining spermatozoa and fertility
- there are two factors that may be involved:
  (a) the effect of the total number of live spermatozoa on fertility
  (b) the interference with fertility by the proportion of dead spermatozoa
- in the dog there has been little study of the proportion of dead spermatozoa within the ejaculate; commonly only the total number of morphologically normal live staining spermatozoa is considered

Spermatozoal motility

- some workers have suggested that spermatozoal motility is the best predictor of male infertility
- human sperm swimming speeds correlate well with the results of in vitro fertilization assays
- measurement of sperm swimming speeds in dogs, and the linearity of direction do correlate with the ability of sperm to penetrate homologous occytes

Cytological studies

- in man much importance has been given to the presence of cells other than spermatozoa within the ejaculate

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- little attention has been given to the presence of these cell types in the dog
- it is common for inflammatory cells to be noted within the ejaculate; however, these frequently originate from the prepuce

Assessment of spermatozoal function

- traditional criteria of semen quality are based upon descriptive assessments of ejaculate characteristics
- however, fertile dogs have been reported with low numbers of normal spermatozoa
- the phenomenon of spermatozoal swelling in the presence of hypo-osmotic medium has been investigated; sperm swelling relies upon a functional membrane which might be a better indicator of fertility than simple live:dead stains which rely upon an intact membrane (see Figure 10.6) [12]
- recent studies have examined computer image analysis of sperm motility and demonstrated that the ability of sperm to change motility characteristics to become hyperactivated correlates with the ability to penetrate homologous oocytes
- oocyte binding assays and oocyte penetration assays are useful for experimental investigation of fertility but as yet are not applicable to routine laboratory use

Treatment of dogs with poor semen quality

- in all cases a holistic approach to the male should be taken, especially with respect to animals that are overweight or obese; dogs should have a well balanced diet with suitable levels of antioxidants
- most medical treatments are unrewarding and the owner should be given realistic advice before embarking on therapy
- an analogue of dihydrotestosterone, mesterolone, is unusual in that it is not aromatized to oestradiol and therefore, at therapeutic doses, does not significantly suppress the release of pituitary gonadotrophins; it may be useful for stimulating spermatogenesis and it has been used in dogs [4], but its effects have not as yet been fully evaluated

General points on infertile dogs

- 'overuse' is rarely a cause of infertility; most dogs produce sufficient spermatozoa for two fertile ejaculations per day; rarely in older dogs daily mating will affect extra-gonadal sperm reserves and the number of sperm ejaculated will decline, and immature sperm (those with distal and proximal cytoplasmic droplets) will appear in the ejaculate
- if semen quality is poor, allow the dog to mate twice daily for as long as the bitch will stand
- if there has been recent illness (pyrexia) it is prudent to rest the dog for 2 months and examine semen after that time. This is not because this will allow

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recuperation, but because the ejaculate will probably be abnormal for this length of time
- do not use androgens (testosterone or its esters) in dogs with poor semen quality or poor libido; these have minimal effect upon libido and generally have a marked suppression effect upon spermatogenesis
- parvovirus vaccination does not cause infertility in male dogs
- dogs with poor semen quality that have immune-mediated thyroiditis usually have systemic signs of hypothyroidism; it is not appropriate to treat with thyroid hormone supplementation 'just in case'
- it should be expected that bacteria will be isolated from the penis and with the exception of Brucella canis there is no known bacterial cause of infertility in dogs

Testicular cysts/spermatocoele

- well defined fluid-filled lesions are not uncommonly identified within the testes using ultrasound examination
- these may be non-significant developmental cysts or be the result of outflow obstruction (a spermatocoele)
- there are no known treatments

Hydrocoele

- these are very rare in the dog
- they may be diagnosed by palpation and ultrasound imaging [3]

Testicular tumours

- testicular tumours are the second most common tumour affecting the male dog
- the author has published ultrasound findings of these tumour types enabling enhanced diagnosis in clinical practice [1,2]
- a variety of different tumour types are seen

Sertoli cell tumours

- these neoplasms are often discrete, slow growing and commonly unilateral
- they generally cause testicular enlargement because of the size of the tumour:
  (a) In the early phases of the disease they are not palpable but they often produce oestrogen and therefore disturb semen quality [2]
  (b) later in the phase of the disease the oestrogen production causes atrophy of normal testicular tissue within the affected testis and the contralateral

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testis; often the contralateral testis is small and the ipsilateral testis is normal sized (because of the neoplasm) or larger than normal
- oestrogen causes feminization and this may be the initial reason for presentation, because the male is attractive to male dogs, or because gynaecomastia, a pendulous prepuce, or bilateral non-pruritic alopecia are noted
- the oestrogen-induced testicular degeneration results in an initial teratozoospermia (many morphologically abnormal sperm) and then azoospermia (absence of sperm from the ejaculate)
- the atrophy of testicular tissue may only be reversible if the tumour is removed within 12 months; after that permanent testicular degeneration ensures
- prolonged oestrogen production may cause bone marrow suppression and results in anaemia and thrombocytopenia
- high oestrogen concentrations may cause squamous metaplasia of the prostate gland
- Sertoli tumours rarely metastasize; only 10% are malignant
- small endocrinologically active tumours may cause clinical signs but be non-palpable; these tumours may be diagnosed ultrasonographically; commonly they are well marginated, have an irregular echogenicity and there is a central zone of fluid representing necrosis
- all tumours should be removed surgically by castration; unilateral castration may result in a return to fertility once the negative feedback effect of oestrogen has been removed

Seminomas

- these tumours arise from spermatogenic tissue of the tubular epithelium
- they are usually solitary and unilateral and cause testicular enlargement which can be very pronounced
- seminomas are usually hormonally inactive, but if they are hormonally active, similar signs and consequences are seen to those with Sertoli cell tumours
- seminomas are most commonly echogenic with ultrasound examination
- these tumours have a medium risk of metastasis and should be removed surgically

Interstitial cell tumours

- these tumours arise from Leydig cells
- they may be single or multiple in one or both testes and are often approximately 1–2 cm in diameter
- affected testicles may shrink due to loss of seminiferous tubules and absence of spermatogenesis and yet surprisingly these tumours are found to be hormonally inactive (or at least do not produce the common hormones seen in Sertoli cell tumours)

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- often not diagnosed due to lack of clinical signs and lack of clinical importance
- yellow in colour when sliced open

NB: Diagnosis of Sertoli cell tumours and seminoma in abdominal testes may only be at exploratory laparotomy by which time metastasis may have occurred.

Feminizing syndrome (see also 20.7)

- most commonly this is associated with a testicular tumour producing oestrogen (see above)
- occasionally the condition is seen in dogs with adrenal oestrogen production
- diagnostic evaluation involves ultrasound examination of the testes and the adrenal glands
- in a small number of cases the condition is seen in dogs with grossly and ultrasonographically normal testes and ultrasonographically normal adrenal glands; anecdotally in some of these dogs castration cures the condition
- feminization may also occur in some castrated dogs, and adrenal steroid production has been implicated
- adrenal disease in either intact or castrated dogs may be evaluated by detecting elevated concentrations of 17-hydroxyprogesterone following a standard ACTH stimulation test
- adrenal disease signs may be controlled by the negative feedback effects of exogenous androgens or progestogens

17.4 EPIDIDYMIDES

- there are few reports of epididymal disease in dogs and much material that is published relates to an assumption that pathology is similar to other species. Obstruction of the epididymis can be diagnosed by absence of the epididymal markers alkaline phosphatase from ejaculated fluid (see 10.6); absence of these may be useful for the diagnosis of obstructive azoospermia, but care must be taken to ensure that complete ejaculation has occurred since dogs that fail to fully ejaculate will also have low concentrations. A small number of conditions have been accurately described
- trauma and inflammation may occur as in the testis
- aplasia has been recorded
- idiopathic blockage; here early in the course of the disease the tail of the epididymis may be noticeably enlarged

17.5 VASA DEFERENTIA

- idiopathic blockage may occur but has not been recorded in dogs
- one case of aplasia has been recorded

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17.6 URETHRA

- there are few abnormalities affecting the prostatic or perineal urethra, although occasionally lesions may develop secondary to urethral obstruction or catheterization; rarely these result in fistula formation
- hypospadias, where the urethra opens in an abnormal position, is usually, but not always, associated with intersexuality (see 18.4)
- urethritis is rare per se but may be associated with urinary calculi, fracture of the os penis and repeated catheterization

Urethral prolapse

- excessive sexual excitement or urinary straining may produce prolapse of the terminal portion of the urethra
- the everted portion becomes oedematous and may bleed; in the acute phase a small red button-like lesion is present on the tip of the penis, later this may become indurated and distorted
- the common presentation is excessive licking at the prepuce
- some dogs show no clinical signs
- early in the course of the disease the prolapsed portion of the urethra may be replaced if not damaged; this is best done using a snug-fitting urethral catheter which needs to be left in place for several days
- amputation of the distal penis may be necessary in chronic cases or when there is marked trauma or the problem is recurrent
- castration should be considered to prevent recurrence

17.7 PROSTATE GLAND

The prostate is the only accessory sexual gland in the male dog.

- prostatic disease is common in the older entire male dog (prostatic neoplasia may occur in the castrated dog). Accurate detection and diagnosis of prostatic disease requires the use of many diagnostic tools (see Chapter 10)
- work in our laboratory has shown that the first and third fractions of the ejaculate originate from the prostate gland [4]; this information is clinically useful since cytological examination of these fluids may reveal the nature of any prostatic pathology

Benign prostatic hyperplasia

- hyperplasia of the prostatic epithelium begins early in the dog's life; both glandular and complex (cystic) hyperplasia occur

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- this is associated with altered androgen:oestrogen ratios; there is a slight decline in androgen concentrations whilst oestrogen concentrations are maintained
- hyperplasia is commonly present without clinical signs
- however, in some dogs the hyperplasia is significant and causes:
  (a) haemospermia
  (b) haemorrhagic urethral discharge
  (c) haematuria
- if the gland continues to enlarge it will impinge upon the pelvic viscera and cause faecal tenesmus; this may lead to rectal diverticula and perineal hernia
- usually the gland is symmetrically enlarged and non-painful; this can be confirmed by digital examination per rectum
- the large prostate gland may be demonstrated radiographically:
  (a) a pneumocystogram may be useful for the accurate identification of the gland
  (b) positive contrast urethrograms may be useful for the assessment of the position of the urethra, and changes within the prostatic urethra
- ultrasonographically there is often enlargement of the gland and slight increased echogenicity (Figure 17.5):
  (a) this change may mask the normal appearance of the hilar echo
  (b) the condition usually progresses quite early to cystic hyperplasia where multiple thin-walled fluid-filled cysts from a few millimetres to several centimetres in size are identified
  (c) later on in the disease the cysts may become very large, and the background texture of the parenchyma becomes quite echogenic and the gland margin may become irregular

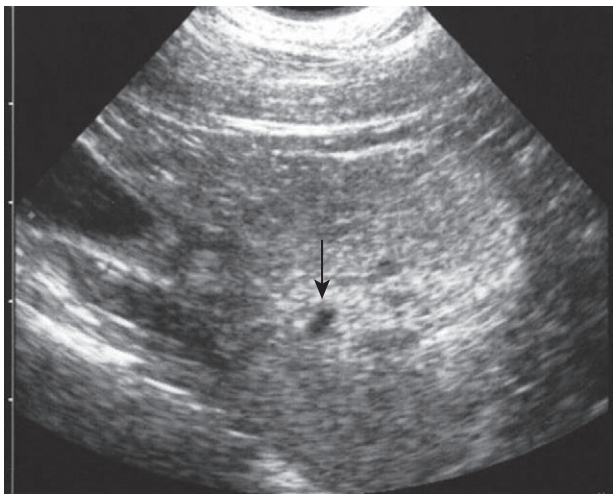


Figure 17.5 Ultrasound image of dog with benign prostatic hyperplasia. There is increased echogenicity of the gland, increased maximal width, and the presence of at least one fluid-filled cyst (arrow).

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- arginine esterase concentrations are elevated in older dogs with prostatic hyperplasia but no data exist for old dogs without hyperplasia; therefore whilst some have described arginine esterase as a specific marker for prostatic hyperplasia as yet this is unconfirmed
- the condition can be managed by removal of androgen stimulation either by castration or medical management
- castration produces rapid resolution of clinical signs and is the treatment of choice for dogs that are not required for breeding
- in stud dogs temporary control of clinical signs may be achieved using either;
  (a) exogenous progestogens – these reduce circulating androgens and have a direct effect on the prostate but do not significantly reduce the size of any cysts; depot therapy (e.g. delvosterone) and oral therapy (e.g. osaterone) is very useful but all progestogens have adverse effects upon semen quality
  (b) 5-alpha reductase inhibitors (e.g. finasteride) prevent the conversion of testosterone into its active form and can be used to reduce prostatic size; daily therapy is required and although there is a substantial effect reducing semen volume there is no effect upon sperm characteristics or fertility
  (c) oestrogens may also be used to reduce circulating androgen concentrations and therefore reduce prostatic size, however these have significant effects upon semen quality and have a range of other adverse effects which make them inappropriate for management of benign prostatic hyperplasia now that finasteride is available

NB: Oestrogens may induce prostatic squamous metaplasia resulting in increased size of the gland.

  (d) GnRH agonists administered via long-lasting implant (e.g. deslorelin) will cause decreased testosterone and shrinkage of the prostate by inducing a reduction in LH secretion

Acute bacterial prostatitis

- adult dogs may develop prostatitis following ascending infection of bacteria along the urethra, commonly these bacteria are Escherichia coli or similar uropathogenic adherent bacteria
- bacterial prostatitis is sometimes a sequel to hyperplasia
- clinical signs include systemic illness, with vomiting and caudal abdominal pain
- dogs are frequently neutrophilic and the gland is painful and has an irregular contour on palpation
- culture of the third fraction of the ejaculate, urethral washing or urine may help with diagnosis
- patchy focal echogenic regions may be identified with ultrasound but early ultrasonographic findings may be equivocal
- treatment of these cases is difficult; antimicrobial therapy on the basis of the results of bacterial sensitivity may be required for up to 6 weeks, progestogens can be useful adjuvant therapy

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- there are particular antibiotics that do not have adverse effects upon semen quality [10]

Chronic bacterial prostatitis

- dogs with acute bacterial prostatitis where no or inadequate treatment is used may develop chronic prostatitis
- bacteria are scattered throughout the gland and pockets of purulent exudate may form
- the gland may become distorted in shape and is often painful to palpate; there may also be attachment of the prostate to the pelvic tissue making it difficult to move
- the prostate is often more echogenic than normal although it may develop a heterogenous appearance as the micro-abscesses become apparent
- large numbers of polymorphonuclear neutrophils and bacteria are present in prostatic fluid collected at the time of ejaculation or following urethral wash
- these cases can be very difficult to treat and require prolonged period of antibiotic administration

Prostatic abscessation

- cases of chronic bacterial prostatitis may progress to prostatic abscessation with multiple pus-filled cavitated lesions
- the prostate becomes very large such that it falls forward into the abdomen and may no longer be palpated within the pelvis per rectum
- prostatomegaly can be detected by radiography or ultrasound; ultrasonographically there are consistent features of thick-walled irregularly septated regions containing fluid with echogenic particles
- these dogs are unable to ejaculate but collection of prostatic fluid by urethral wash reveals large numbers of bacteria and polymorphonuclear neutrophils
- the consistent bacterial focus in the urethra often produces signs of recurrent cystitis
- surgical ablation or omentalization is required combined with a prolonged treatment with antimicrobial agents

Squamous prostatic metaplasia

- a change in the prostatic glandular epithelium from cuboidal or columnar to squamous occurs secondarily to endogenous or exogenous hyperoestrogenism (e.g. Sertoli cell tumours)
- this change predisposes to cyst formation, infection and abscessation, but is not a pre-neoplastic change
- a presumptive diagnosis may be made on the history and clinical signs

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- treatment is directed towards removal of the source of oestrogen because the condition is reversible

Prostatic cysts

- two types of prostatic cyst have been identified:
  (a) the least common are discrete retention cysts associated with blockage of the prostatic ducts; these occur within the parenchyma of the prostate, causing distortion of its outline
  (b) more commonly, cysts are found adjacent to the prostate (paraprostatic cysts), attached by small stalk-like adhesions; these are remnants of the uterus masculinus
- the clinical signs of both types of cyst are related to their size; they may produce pelvic canal obstruction, faecal tenesmus, dysuria and haematuria
- diagnosis may be made radiographically, although ultrasound examination can differentiate the internal architecture of the organ:
  (a) paraprostatic cysts are frequently large anechoic structures positioned cranial or dorso-cranial to the bladder
  (b) the removal of urine or injection of small volumes of air into the bladder may allow differentiation from the paraprostatic cyst in cases of doubt
  (c) the cyst wall can be of variable thickness and may be echogenic due to calcification
  (d) cyst fluid may contain echogenic material which often sediments to the dependent portion
  (e) many cysts have echogenic internal septae and a connection to the prostate gland may sometimes be imaged
  (f) it is not uncommon for cystic lesions to also be identified within the prostate gland (Figure 17.6)

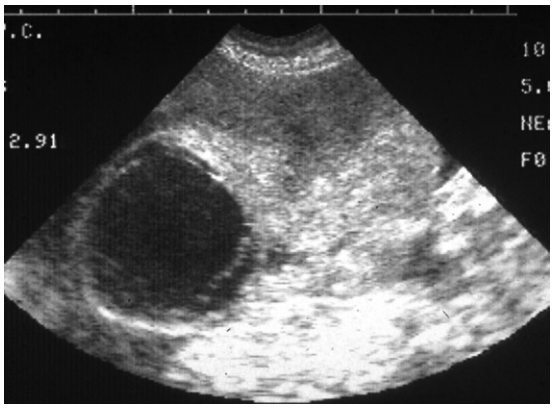


Figure 17.6 Ultrasound image of dog with paraprostatic cyst. There is a central region anechoic fluid containing echogenic particles, the wall is irregular and thickened and in some zones is calcified.

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- excision or marsupialization are the treatments of choice; castration and hormone therapy are of no value

Prostatic neoplasia

- adenocarcinomata are the most common neoplasms affecting the prostate gland of the dog (Figure 17.7)
- tumours are firm upon palpation and tend to metastasize to iliac and sublumbar lymph nodes and to the vertebral bodies of caudal lumbar vertebrae
- clinical signs are associated with the increased size of the gland, although dogs may have a haemorrhagic urethral discharge and hind limb pain
- diagnosis may be made on the basis of clinical signs, radiographic findings, the presence of neoplastic cells collected by urethral washing following prostatic massage or semen collection
- patchy focal regions of mixed echogenicity may be identified with ultrasound; these may be associated with an increased parenchymal echogenicity and fine echogenic speckles, overall there is loss of the normal architecture
- our work has shown that it is possible to assess prostatic perfusion using colour-enhanced ultrasound; using this technique we have demonstrated differences in the perfusion of lesions that are malignant from those that are benign [13,14]; these techniques will in the future be developed further to enhance our diagnostic ability in clinical practice
- fine-needle aspiration under ultrasound guidance or biopsy are valuable diagnostic tools
- the animal should be carefully examined for metastatic spread before embarking on therapy
- treatment can be palliative by the administration of oestrogens, anti-androgens, and by castration

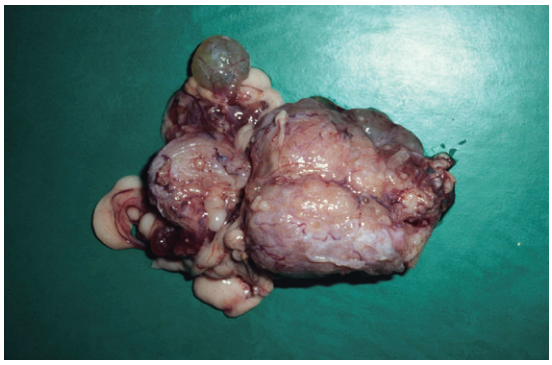


Figure 17.7 Prostatic adenocarcinoma.

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Medical therapy for prostatic disease (see also Chapter 27)

- long-term therapy is aimed at reducing the size of the gland, i.e. reducing circulating concentrations of androgens
- this can be achieved by the repeated administration of hormone preparations:
  (a) progestogens which have a negative feedback effect upon the pituitary (there are available as daily oral or injectable depot preparations)
  (b) finasteride which is a specific 5-alpha reductase inhibitor which prevents the conversion of testosterone to dihydrotestosterone
  (c) oestrogens which have a negative feedback effect upon the pituitary but suffer the risk of inducing prostatic metaplasia
  (d) flutamide which inhibits androgen uptake and/or nuclear binding of androgen
  (e) formestane which is an aromatase inhibitor which inhibits the conversion of androgen to oestrogen in peripheral tissue

NB: No medical therapies will produce permanent relief of large prostatic cysts or neoplasia.

- antibiotics which are chosen for chronic prostatitis and prostatic abscessation should be chosen upon the basis of specific sensitivity; however, erythromycin, chloramphenicol and trimethoprim are most likely to be concentrated in prostatic fluid, although other agents may penetrate the gland when it is inflamed
- analgesics and anti-inflammatory drugs may be indicated

Surgical removal of the whole gland, a portion of the gland, a cystic region or abscessed area may be considered.

Effects of prostatic disease on fertility

- blood and inflammatory cells in the ejaculate do not seem to markedly affect fertility although substantial sperm clumping may occur
- it is possible that changes in the prostatic fluid that occur in cases of prostatic disease may have osmotic or other effects on sperm which reduce their viability
- medical treatments with progestogens or oestrogens inhibit testicular and prostatic function temporarily; after treatment fertility may return but so may clinical signs of disease
- medical treatment with finasteride reduces the volume of the ejaculate but does not change semen quality or fertility
- castration and prostate removal cause sterility

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17.8 SCROTUM

Scrotal trauma (abrasions, eczema)

- any trauma to the scrotum is painful and results in substantial licking which frequently causes additional secondary trauma
- immediate treatment (antibiotics, analgesics, Elizabethan collar) is necessary to bring testicular temperature under control to ensure that there are no secondary testicular changes (testicular degeneration) (see 17.3)

Inguinal (scrotal) hernia

- this is rare in the dog and is recognized as swelling in the scrotum due to the passage of abdominal contents through the inguinal canal
- treatment is surgical correction, castration and closure of the inguinal canal

17.9 MAMMARY GLAND

Mammary gland disease is rare in the dog but mammary tumours do occur; most are benign and the principles of treatment are as for those described in the bitch (see 16.8).

REFERENCES

1 England, G.C.W. (1991) The relationship between ultrasonographic appearance, testicular size, spermatozoal output and testicular lesions in the dog. *Journal of Small Animal Practice* **32**, 306–311.
2 England, G.C.W. (1995) Ultrasonographic diagnosis of non-palpalable Sertoli cell tumours in infertile dogs. *Journal of Small Animal Practice* **36**, 476–480.
3 Ahmad, N., England, G.C.W. and Noakes, D.E.N. (2000) Sequential ultrasonography of spontaneously-occurring lesions of the genital system of rams, and their influence on semen quality. *Veterinary Record* **146**, 10–15.
4 England, G.C.W., Allen, W.E. and Middleton, D.J. (1990) An investigation into the origin of the first fraction of the canine ejaculate. *Research in Veterinary Science* **49**, 66–70.
5 England, G.C.W., Allen, W.E. and Porter, D.J. (1989) The evaluation of the testosterone response to human chorionic gonadotrophin, and the identification of a presumed anorchid dog. *Journal of Small Animal Practice* **30**, 441–443.
6 England, G.C.W. (1995) Evaluation of seminal characteristics in the dog. *Journal of Animal Breeding* **1**, 38–45.
7 England, G.C.W. and Allen, W.E. (1993) The effect of sulphasalazine upon seminal characteristics of dogs. *Journal of Reproduction and Fertility* **47**(Supplement), 526–528.
8 England, G.C.W. and Allen, W.E. (1991) The effect of the synthetic androgen mesterolone upon seminal characteristics of dogs. *Journal of Small Animal Practice* **32**, 271–274.

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9 England, G.C.W. and Allen, W.E. (1991) The lack of effect of parvovirus vaccination upon seminal characteristics of dogs. *Veterinary Record* **128**, 611–612.
10 England, G.C.W. (1999) Effect of clavulanic acid-potentiated amoxycillin on semen quality in dogs. *Theriogenology* **52**, 1117–1122.
11 England, G.C.W. and Allen, W.E. (1989) Fertility and semen parameters in dogs. *Veterinary Record* **125**, 399.
12 England, G.C.W. and Plummer, J.M. (1993) Hypo-osmotic swelling of dog spermatozoa. *Journal of Reproduction and Fertility* **47**(Supplement), 261–270.
13 Russo, M., Vignoli, M., Catone, G., Rossi, F., Attanasi, G. and England, G.C.W. (2009) Prostatic perfusion in the dog using contrast-enhanced doppler ultrasound. *Reproduction in Domestic Animals* **44**, 334–335.
14 Vignoli, M., Russo, M., Catone, G., Rossi, F., Attanasi, G., Terragni, R., Saunders, J.H. and England G.C.W. (2011) Assessment of vascular perfusion kinetics using contrast-enhanced ultrasound for the diagnosis of prostatic disease in dogs. *Reproduction in Domestic Animals* **46**, 209–213.

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18 Intersex Animals

Whilst intersex animals are not that common, the clinical presentation results in substantial confusion warranting a separate chapter in this book. Intersex animals are most commonly recognized because of abnormal external (phenotypical) sex appearance.

18.1 DEFINITIONS

True hermaphrodite: in mammals this word is used to describe individuals which have both testicular and ovarian tissue; invariably the testicular tissue is intra-abdominal so that spermatogenesis does not occur.

Male pseudohermaphrodite: this is an animal with testicular tissue but external genitalia which are to a greater or lesser extent 'female-like' (Figure 18.1a).

Female pseudohermaphrodite: has ovarian tissue but external genitalia which are to a greater or lesser extent 'male-like' (Figure 18.1b).

Intersex: this is an individual in which the physical features do not conform to those generally recognized as belonging to the male or female. This is the best word to use to describe dogs of indeterminate sex as their gonadal content will not be known until histological examination is made (for clarity intersex animals may be true hermaphrodite or pseudohermaphrodite).

18.2 AETIOLOGY

Intersex animals may be classified into those with abnormalities of either (1) chromosomal, (2) gonadal or (3) phenotypic sex.

Abnormalities of chromosomal sex (see 1.6 for basic genetics)

- generally these animals are phenotypically male or female but they have underdeveloped genitalia

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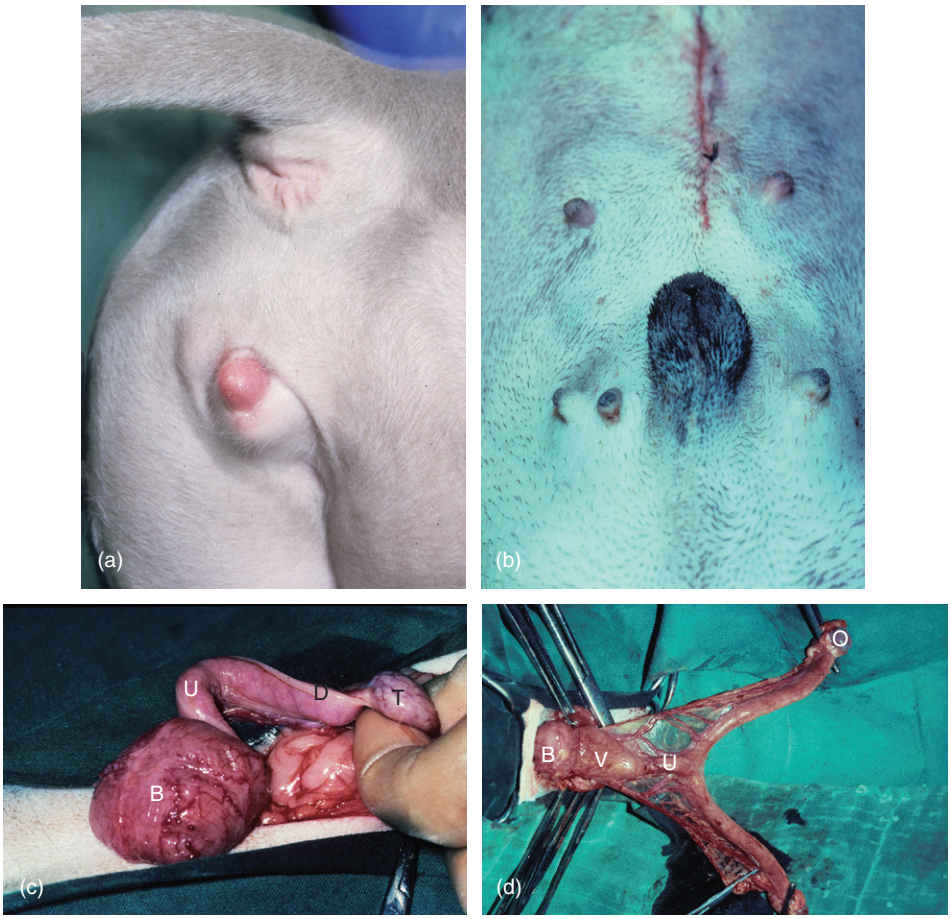


Figure 18.1 Intersex dogs. (a) This animal was sold as a normal female pup; at 5–6 months of age, growth and exposure of the phallus (clitoris) was noticed. (b) This dog was assumed to be a normal male until he was presented with haemorrhage from the prepuce and a history of being attractive to male dogs; closer examination revealed that the prepuce was rudimentary, there was no palpable penis and the dog was in oestrus. (c) Internal genitalia from dog in (a); a uterine horn (U), ductus deferens (D) and testis (T) are shown (B, urinary bladder). (d) Internal genitalia from dog in (b); the vagina (V), uterus (U) and ovaries (O) are shown (B, urinary bladder).

- accurate demonstration of chromosomal abnormalities requires construction of a karyotype (a blood sample is sent to an appropriate diagnostic laboratory)
- the chromosomal abnormalities are not usually an inherited condition, rather this can be:
  (a) an extra sex chromosome, e.g. XXY, XXX
  (b) only one sex chromosome, e.g. XO
  (c) more than one cell line in the same individual with different sex chromosome contents (termed a 'mosaic'), e.g. XX/XY or XX/XXY

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Abnormalities of gonadal sex

- these animals may initially appear to have normal genitalia but whilst they have a normal chromosome constitution the chromosomal and gonadal sex do not agree
- affected animal are said to be 'sex-reversed'
- only XX sex reversal is seen in dogs, i.e. XX male or XX pseudohermaphrodite
- such dogs have testes or ovotestes
- this is seen as an inherited condition of cocker spaniels and other breeds
- it is thought to be caused by an inherited sex-reversal gene

Abnormalities of phenotypic sex

- these animals have an intermediate appearance to the external genitalia and yet the chromosomal and gonadal sex agree
- clearly that has to have been some problem in the development of the reproductive tract
- female pseudohermaphrodites are caused by exposure of the fetus to sex steroids (usually androgens or progestogens) during critical stages of development; usually these are exogenously administered but sometimes one fetus is affected by an adjacent fetus or the dam has a hormonal imbalance
- male pseudohermaphroditism may be inherited or be due to a defect in androgen production

18.3 CLINICAL SIGNS

- affected individuals are usually not recognized as puppies
- in some cases the external appearance is normal or nearly normal and no abnormality is noticed until either breeding is proposed or until surgical neutering
- in many cases abnormality is noticed around the time of puberty; commonly, abnormal development of the genitalia, particularly enlargement of the clitoris (phallus), is noticed at about 6 months of age
- other signs which alert an owner to abnormality include pro-oestrous bleeding in dogs thought to be males, and urinary incontinence
- incontinence is usually caused when a normal vagina opens into a severely narrowed vestibule associated with an enlarged os clitoridis (Figure 18.1a); urine is then voided normally from the bladder but accumulates in the distended vagina from which it leaks slowly via the stenosed vestibule
- adult dogs, particularly male pseudohermaphrodites, may first be presented with pyometra or Sertoli cell tumour

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18.4 EXTERNAL PHYSICAL SIGNS

- in dogs that look mainly female, the vulva is more ventrally placed than usual and may be between the thighs; there may be varying protrusion of the clitoris due to enlargement of the os clitoridis which now resembles a small os penis with a narrowed vestibular canal running like a male urethra along its caudal surface
- in dogs that look mainly male-like the preputial opening is further forward but the prepuce is short; the preputial opening may be ventral with urethral hypospadias (see 17.2) – alternatively the prepuce may be normal but contains no penis
- if testicles are present in either of the above they may be abdominal or scrotal

18.5 INTERNAL REPRODUCTIVE ORGANS

- irrespective of the external signs the gonads may be ovaries, testes or a mixture of both (ovotestes); they may be in a normal ovarian position, or located anywhere along the normal testis path of descent
- irrespective of the sort of gonads present, the tubular genitalia may consist of a uterus, or epididymis/vas deferens, or both together

18.6 TREATMENT

- in terms of prognosis intersex dogs are nearly always sterile and therefore treatment is aimed at producing a pet with minimal complications from the underlying abnormality
- removal of the gonads and tubular genitalia at laparotomy prevents the discomfort that some animals show in oestrus, avoids pyometra and prevents the development of testicular tumours
- after removal of testicular tissue from dogs with a protruding phallus, time should be allowed for this to regress; otherwise reduction of the size of the phallus by removing the os clitoridis/penis may be necessary
- there may be no cure for incontinence in intersexual dogs, although a variety of reconstructive techniques have been described but are not in the scope of this book

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19 Abnormal Female Physiology and Endocrinology

There are many different causes of infertility and reproductive disease in the bitch; physiological and endocrinological abnormalities are important and reference to the normal physiology can often elucidate the exact abnormality.

19.1 DELAYED PUBERTY (PRIMARY ANOESTRUS)

- the age of a bitch at her first pro-oestrus/oestrus is very variable; it may not occur until she is over 2 years of age
- the reason for this delay is usually not known but it is clear that large and giant breeds of dog reach puberty later than smaller breeds; often puberty is reached when the female reaches approximately 80% of her adult body mass
- it is not uncommon for some bitches to be very fastidious at the first oestrus and to lick away any vulval discharge such that the pubertal oestrus is overlooked by the owner
- when pro-oestrus/oestrus does eventually occur, it is usually normal
- there is no reason why a mature bitch should not be mated at her first oestrus; indeed several hereditary diseases are not present in the female at a young age (e.g. many eye diseases) and therefore there is no rush to breed bitches early
- the best approach is not to attempt oestrus induction but to monitor vaginal cytology so that the onset of pro-oestrus can be predicted, or to measure plasma progesterone concentration; an elevated progesterone concentration indicates that the oestrus has been missed by the owner
- if oestrus has not been missed and the delay is causing concern, establishment of the karyotype can be useful in some cases prior to attempting oestrus induction (see 24.4)
- if signs of pro-oestrus/oestrus are induced this at least confirms that the bitch has an ovary and that it can respond to gonadotrophic stimuli
- a dog with X-chromosome trisomy and elevated follicle-stimulating hormone (FSH) and luteinizing hormone (LH) plasma concentrations has been reported; it was still anoestrus at 4 years old
- hypothyroidism is rarely the cause primary anoestrus

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19.2 PROLONGED FIRST PRO-OESTRUS

- in some bitches the first pro-oestrus does not proceed to oestrus and may last for 3 weeks or more, or pro-oestrus may terminate and recur some weeks later
- presumably there is insufficient pituitary stimulation to cause follicle maturation or insufficient LH receptors on the follicles
- as a result ovulation does not occur, follicles therefore regress and signs of pro-oestrus disappear
- theoretically, administration of human chorionic gonadotrophin (hCG – which is LH-like in activity) should cause ovulation; however this is rarely effective which suggests that follicles are still immature
- often a second follicular phase appears (2–12 weeks later) and the bitch ovulates normally; very rarely the anovulatory cycle is repeated before a third ovulatory cycle
- when the signs of pro-oestrus are very prolonged (more than 45 days) and hCG treatment has been unsuccessful it may be prudent to suppress the signs of oestrus using a progestogen (megestrol acetate at 2 mg/kg for 8 days usually causes cessation of pro-oestrus); the time of occurrence of the next pro-oestrus is variable but it usually then proceeds normally

NB: There is some risk of inducing a pyometra by administration of a progestogen when oestrogen concentrations are elevated.

Very rarely young bitches with persistent pro-oestrus have ovarian tumours that produce oestrogen; these can relatively easily be diagnosed by ultrasound examination.

19.3 PROLONGED PRO-OESTRUS OR OESTRUS (Figure 19.1)

- it is important to recognize that normal bitches may not ovulate at the expected time; in the author's clinic we found that some normal fertile bitches do not ovulate until 30 days after the onset of pro-oestrus [1]; these data are important and have enabled differentiation of the normal from the abnormal bitch in clinical practice
- a condition similar to prolonged first pro-oestrus (see 19.2) may occur in the adult bitch that has previously been normal
- the condition appears to be most common in German shepherd dogs and similar breeds as part of an anovulation/short luteal phase syndrome
- in these cases administration of hCG (500 IU/bitch on 3 consecutive days) may be useful to 'force' ovulation; a key consideration is the time that hCG is administered and it appears best when there is maximal cornification of vaginal epithelial cells or when there is a slight increase in plasma progesterone concentration

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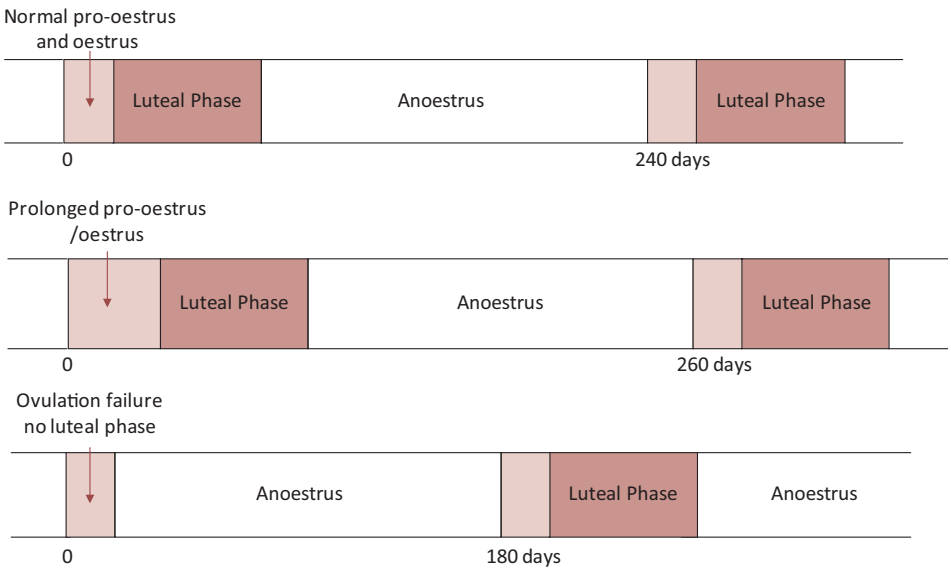


Figure 19.1 Schematic representation of return to cyclicity in days for bitches with prolonged pro-oestrus/oestrus and bitches with ovulation failure.

- despite induction of ovulation some of these bitches have a short luteal phase and if pregnant may resorb or abort; administration of progestogens throughout pregnancy has been described but must be applied cautiously otherwise it will affect the sexual development of the pups in utero

19.4 SHORT INTER-OESTROUS PERIODS (OVULATION FAILURE) (Figure 19.2)

- intervals of less than 4 months between successive oestrous periods suggest that:
  (a) ovulation (luteinization) may not have occurred
  (b) the luteal phase may be short; this has only been reported after oestrus induction and in the German shepherd and similar breeds (see 19.3)
- a difficulty with the condition is that the bitch may have a normal length pro-oestrus and oestrus and when these end the cycle is thought to have been normal (although there has been no ovulation)
- the condition is then recognized when the bitch does not become pregnant and there is a short interval to the next pro-oestrus
- confirmation of ovulation can however be achieved by monitoring plasma progesterone concentrations
- the length of the luteal phase can be monitored by regular measurement of plasma progesterone concentration (see 5.9)

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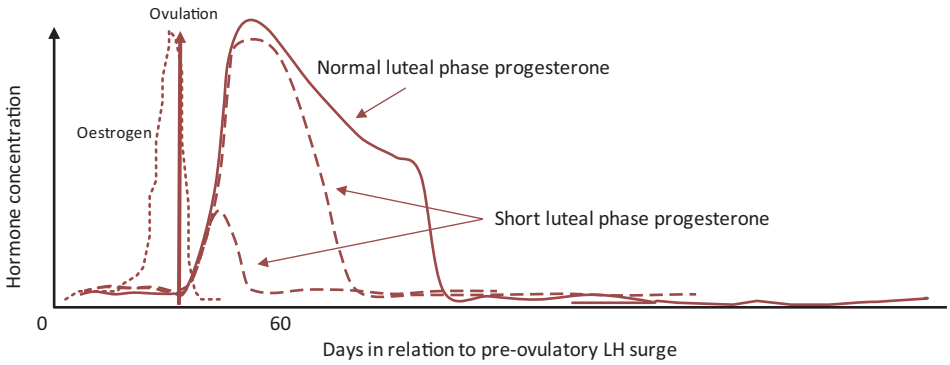


Figure 19.2 Schematic representation of short luteal phase seen in German shepherd and similar breeds where there is abnormal ovulation/inadequate development of the corpora lutea

- in cases of anovulation, an attempt to induce ovulation can be made by the administration of hCG, but
  (a) it is difficult to know when to administer this
  (b) early administration may result in follicular luteinization
  (c) late administration may be ineffectual
- administration is best planned at maximal cornification of vaginal epithelial cells or when there is a slight increase in plasma progesterone
- the short luteal phase can result in pregnancy but subsequent loss as progesterone concentration decreases, or lack of pregnancy (Figure 19.2)

19.5 PROLONGED ANOESTRUS (SECONDARY ANOESTRUS)

- the inter-oestrous interval may normally be longer than expected:
  (a) in many cases this is because pro-oestrus has not been observed by the owner but occurred normally; elevated concentrations of progesterone (greater than 2 ng/ml) will indicate a missed ovulation within the previous 2 months
  (b) in some bitches there is systemic illness for example and endocrinopathy that prevents the return to pro-oestrus; rarely this may be associated with luteal cysts; hypothyroidism does not appear to be a common cause of prolonged anoestrus unless there are very marked clinical signs
  (c) some drugs including progestogens, androgens, glucocorticoids and anabolic steroids have the ability to prevent oestrus
  (d) in the basenji dog oestrus occurs only every 12 months
- if the delayed return to pro-oestrus interferes with a breeding programme, oestrus induction may be considered (see 24.4)

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19.6 UNPREDICTABLE OVULATION TIME (see also 3.4 and Figure 3.2)

- in normal bitches there is a very large variation in the day on which ovulation occurs after the onset of pro-oestrus; this constitutes the most common cause of infertility since often the owner decides which day to attempt breeding, and usually they choose a fixed day, e.g. day 12 and day 14
- whilst the 'average bitch' ovulates approximately 12 days after the beginning of pro-oestrus, this is not true for all bitches:
  (a) some bitches ovulate as early as day 5 after the onset of pro-oestrus
  (b) some bitches ovulate as late as day 30 after the onset of pro-oestrus
- such bitches are normal but they are not 'average'
- bitches are not necessarily consistent at successive heats; some bitches may vary by as much as 12 days in the day of ovulation between successive cycles, i.e. they ovulate on day 8 at one oestrus and day 20 at the subsequent oestrus [1]
- careful monitoring of each oestrous cycle is therefore warranted; this can be achieved using vaginal cytology, vaginal endoscopy and measurement of plasma progesterone concentration

19.7 SILENT OESTRUS

- some bitches have normal cyclical activity, including follicular growth and ovulation, without any external signs of pro-oestrus or oestrus
- vulval swelling appears minimal during oestrus; these bitches are also very fastidious and clear away any vulval discharge by licking
- ovulation may be confirmed by examination of plasma progesterone concentration
- alternatively, the weekly collection of cells from the bitch's vagina will allow anticipation of oestrus
- demonstration of an elevated progesterone concentration will demonstrate that oestrus has occurred within the last 2 months and was not observed by the owner

19.8 PSEUDOPREGNANCY (see also 3.7)

- all non-pregnant bitches will develop mammary gland enlargement and the production of milk during the late luteal phase
- many non-pregnant bitches exhibit signs which are similar to those seen normally during the pre-partum and post-partum periods
- signs may develop occasionally, or following every oestrus in an individual bitch
- the clinical signs of pseudopregnancy are not associated with any endocrine abnormality; they are related to a normal elevated plasma concentration of prolactin

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- the term physiological pseudopregnancy may be used in some cases, because in every non-pregnant bitch there is some degree of mammary gland enlargement

Clinical signs

- clinical pseudopregnancy (sometimes referred to as overt pseudopregnancy) has a variable clinical presentation including anorexia, nervousness, aggression, nest making, nursing of inanimate objects, lactation and occasionally false parturition
- in some cases lactation may result in mastitis
- pseudopregnancy is generally noted between 6 and 12 weeks after the end of oestrus, during the decline in plasma progesterone concentration
- it may also occur should plasma progesterone concentration decline for other reasons:
  (a) removal of the ovaries (i.e. ovariectomy or ovariohysterectomy) during the luteal phase will result in a rapid reduction in plasma concentration of progesterone, and a subsequent rise in plasma concentration of prolactin
  (b) lysis of the corpora lutea may result in a rise in plasma prolactin concentration, i.e. following spontaneous abortion in late pregnancy, or the pharmacological termination of pregnancy
- in many cases pseudopregnancy undergoes spontaneous remission, especially if there is no stimulus for continued lactation (no sucking)
- in some cases either the physical or the psychological signs, or the effect of these signs upon the owner, may warrant treatment of the condition
- in some cases pseudopregnancy may persist for many months, or even several years; the reason for this is unknown, but presumably relates to a lack of hormonal interaction between the pituitary/hypothalamus and the ovary
- it is often mistakenly stated that clinical pseudopregnancy predisposes to pyometra, infertility and ovarian abnormalities; however, this is not the case

Treatment

- in the majority of cases, clinical pseudopregnancy requires no treatment; spontaneous resolution can be allowed to occur
- ovariectomy or ovariohysterectomy has no value for the treatment of clinical pseudopregnancy:
  (a) at certain stages of the cycle removal of the ovaries will cause a further reduction in plasma progesterone, and potentiate the rise of plasma prolactin
  (b) ovariohysterectomy will prevent the occurrence of pseudopregnancy, but caution should be exercised in performing surgery too soon after recovery
  (c) in bitches that have repeated clinical pseudopregnancy the best time to perform surgery is during the subsequent oestrus

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Conservative treatment

- anecdotally it is reported that increasing exercise and possibly reducing food intake may be helpful
- sedative agents may be useful, but phenothiazines may be inappropriate, because they are dopamine antagonists and may produce a rise in prolactin concentration
- massage of the mammary glands should be avoided because stimulation may potentiate a further rise in prolactin concentration
- diuretic agents may speed the resolution of the clinical signs but should be administered with care, especially if the bitch is anorexic

Drug therapy

- before embarking upon medical treatment it is important to establish that the bitch is pseudopregnant and not pregnant, because administration of pharmaceutical products used to treat pseudopregnancy to a pregnant bitch will terminate pregnancy or prevent parturition
- we have documented exactly this case scenario when bitches presented as pseudopregnant were in fact pregnant despite the owners being adamant that the bitch could not have been mated [2]

Prolactin inhibitors

- several dopamine agonists have been shown to be inhibitors of prolactin secretion
- cabergoline is widely available and has few adverse effects compared with previous prolactin inhibitors
- cabergoline may cause vomiting in a small proportion of cases (it is best administered with food), it may also cause a more rapid return to pro-oestrus than anticipated and if inadvertently administered to a pregnant bitch it will cause pregnancy termination
- prolactin concentrations decrease rapidly after administration and the behavioural manifestations reduce quickly and milk production decreases a few days later
- treatment is usually administered for 5 or 7 days or longer if signs remain at the end of planned treatment

Progestogens

- progesterone (or progestogen) use has been largely superseded by the prolactin inhibitors
- progestogens produce a reduction in concentrations of prolactin and rapidly reduce the clinical signs
- progestogens may be administered either orally daily (e.g. megestrol acetate), or by depot injection (e.g. proligestone or delmadinone acetate)
- first-generation progestogens such as medroxyprogesterone acetate are not recommended because they might potentiate the development of a pyometra

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- rapid cessation of oral therapy is often associated with relapse of the clinical signs, and gradual reduction of the dose over 7 days is recommended to prevent this

Oestrogens and androgens

- both oestrogens and androgens alone and in combination are effective at reducing the clinical signs of clinical pseudopregnancy
- their mechanism is likely to be the same as that of progesterone (i.e. inhibition of prolactin concentrations)
- androgens or oestrogens are not frequently used alone; however, a synthetic androgen, mibolerone, which is not available in the UK but is available in the USA, is effective
- in some countries combinations of ethinyl oestradiol and methyltestosterone were manufactured to treat clinical pseudopregnancy in the bitch and produced a good clinical response and minimal adverse effects, however they are no longer commercially available

19.9 THYROID DEFICIENCY

- hypothyroidism is said to cause infertility occasionally in the bitch
- this may be via a delayed return to oestrus but in the author's experience this is never the case unless there are clear clinical manifestations of hypothyroidism; assessment of thyroid hormone profiles in clinically normal bitches is therefore not warranted
- lymphocytic thyroiditis has been identified as a cause of infertility in beagles and borzois
- there is no evidence that thyroid supplementation improves fertility in clinically normal bitches

19.10 OTHER HORMONAL ABNORMALITIES

- to date there have been few descriptions of hormone abnormalities which cause unusual behaviour or conditions in the bitch because:
  (a) the only hormones readily measured in the bitch are progesterone and oestrogen
  (b) hormonal changes are so rapid that frequent sampling would be necessary but impracticable; the assay of single samples is usually worthless (except progesterone, see 5.9)
- terms such as 'ovarian imbalance' and 'hormonal imbalance' are meaningless because they cannot be defined

Conditions thought to be caused by hormonal abnormality

Mounting and thrusting behaviour

- this occurs in both entire and neutered bitches
- it may be more marked when other oestrous bitches are present

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- there is no evidence that it is caused by an excess of androgens or any other specific hormone change
- it can be controlled by training

Urine marking

- during pro-oestrus and oestrus it is common for bitches to pass small volumes of urine frequently; they may also urinate on vertical objects
- bitches may also do this when not in oestrus and even after neutering; it does not indicate a hormonal anomaly

Urinary incontinence (see 26.1)

- this may occur in older bitches which have been neutered
- such bitches do not have lower oestrogen concentrations than other neutered bitches (within the limits of sensitivity of the assays available)
- the reason why only some neutered bitches are affected is not known but may relate to differences in gonadotrophin receptors within the urethra
- many bitches respond to low-dose oestrogen therapy (see 27.2) or to drugs which cause urethral muscle contraction (e.g. phenylpropanolamine)

Oestrogen-responsive dermatosis

- some neutered bitches develop a bilateral, non-pruritic alopecia, usually with hyperpigmentation of the skin
- such bitches cannot be said to be hypo-oestrogenic because plasma concentrations are in the same range as other neutered bitches

Unwillingness to mate during oestrus

- some bitches which are considered to be in oestrus by virtue of vaginal and other changes will not allow mating
- hormone concentrations have not been reported but are probably within the normal range
- the condition is usually psychological and seen in bitches which have had little contact with other dogs
- treatments include tranquillizers, exposure to a different male dog and artificial insemination

REFERENCES

1 England, G.C.W., Allen, W.E. and Blythe, S.A. (1989) Variability of the time of calculated luteinising hormone release in 218 canine pregnancies. *Veterinary Record* **125**, 624–625.

2 England, G.C.W (1998) Complications of treating presumed pseudopregnancy in pregnant bitches. *Veterinary Record* **142**, 369–371.

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20 Abnormal Male Physiology and Endocrinology

20.1 HYPOGONADISM

- rarely seen but a congenital (present at birth) problem of reduced secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) which results in impaired spermatogenesis
- may occur in cases of pituitary dysfunction
- classical features are the very small size of the testes compared with the body mass of the dog
- serial blood sampling demonstrates that plasma LH, FSH and testosterone concentrations are low
- testicular fine-needle aspiration demonstrates an absence of spermatogenesis but no inflammation
- in some species the administration of FSH has been suggested to promote spermatogenesis although the efficacy of this technique remains to be proven; it has never been documented in dogs and the prognosis for fertility is hopeless

20.2 POOR LIBIDO

- an infrequent problem manifest as an uninterest or unwillingness to copulate
- unwillingness to copulate despite interest in the female is most commonly the result of a previous poor experience at breeding, inexperience on behalf of the dog or poor breeding management
- uninterest may be present in cases of hypogonadism and small testes will be apparent
- there is no evidence that in normal dogs reduced libido is the result of low plasma androgen concentrations
- the administration of exogenous androgens is rarely curative, and importantly testosterone and other androgens will have an inhibitory effect upon pituitary function resulting in reduced FSH secretion and a subsequent reduction in semen quality [1]; androgens and anabolic steroids are contraindicated in breeding male

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20.3 ABSENCE OF SPERMATOGENESIS

- the vast majority of cases seen in practice are a result of a previous insult to the testes, i.e. these are cases of testicular degeneration/spermatogenic arrest
- an impaired or absent spermatogenesis is seen in cases of hypothyroidism
- in other species a variety of other primary causes of absence of spermatogenesis are reported, including primary testicular failure, primary failure of spermatogenesis and Leydig cell failure; the occurrence of these conditions in the dog is entirely speculation
- if a primary testicular abnormality were present then basal concentrations of FSH and LH would be high
- if a primary pituitary or hypothalamic abnormality were present then FSH and LH concentrations would be low
- it is also possible that low concentrations of plasma LH, FSH and testosterone may occur secondarily to thyroid hormone deficiency
- histologically, the testes of many azoospermic dogs have tubular degeneration, with normal or sparse numbers of Leydig cells
- ejaculates contain no sperm, but where there is Leydig cell failure there will be low androgen concentrations and these dogs do not ejaculate
- it is possible that the synthetic androgen, mesterolone, may be useful in these cases [2]
- thyroid function should always be evaluated in cases of poor semen quality: cases of hypothyroidism may benefit from supplementation; however, the prognosis for fertility is often poor

20.4 OTHER CAUSES OF ABNORMAL SPERMATOGENESIS

- a variety of conditions may result in abnormal spermatogenesis (see 17.3):
  - (a) testicular trauma resulting in both tissue damage and elevated temperature
  - (b) testicular tumours resulting in endocrine changes and subsequent reduced spermatogenesis
  - (c) testicular ectopia resulting in increased testicular temperature
  - (d) systemic hyperthermia
  - (e) aplasia of testis or epididymis
  - (f) scrotal trauma or eczema resulting in elevated temperature
  - (g) testicular degeneration after orchitis, torsion or other inflammatory conditions
  - (h) autoimmunity resulting in spermatogenic arrest
  - (i) unknown causes of spermatogenic arrest
  - (j) endogenous endocrine disturbances
  - (k) exogenous hormone administration (progestogens, oestrogens and androgens; which may have been given to control sex drive, control prostatic disease or promote libido) [1]

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- raised FSH and LH plasma concentrations have been reported in dogs with spermatogenic arrest because of failure to respond to stimulation resulting in a reduced feedback effect

20.5 IMPAIRED SPERMATOZOAL TRANSPORT

Accelerated passage through the epididymis

- in some species it is known that overuse results in accelerated transport of sperm through the epididymis and the presence of immature sperm in the ejaculate
- normal healthy dogs can cope with one ejaculation per day for prolonged periods of time without significant impact upon sperm morphology although the number of sperm ejaculated will decrease to a stable number called the daily sperm production
- short periods of time with a higher ejaculatory frequency or dogs with marginal semen quality mating more than 5 times per week may result in immature sperm appearing within the ejaculate
- the immature sperm have proximal cytoplasmic droplets and generally are thought not to be able to fertilize
- small numbers of sperm with distal cytoplasmic droplets may represent accelerated transport but this is not uncommonly seen in dogs with a normal mating regime

Retrograde ejaculation

- some dogs have an absence of sperm within the ejaculate and usually a small ejaculate volume, this may appear at odds with the fact that there are normal urethral contractions during semen collection
- catheterization of the bladder and microscopic examination of urine demonstrates a large number of sperm which is the result of retrograde ejaculation, presumably the result of an incompetent bladder sphincter or uncoordinated urethral contractions
- sperm present within the bladder are often immotile, but may return to motility and fertility after dilution with physiological saline, centrifugation, removal of the supernatant and re-suspension
- treatment may be attempted using sympathomimetic agents which aim to increase bladder sphincter tone

NB: It is common to find low numbers of sperm within the urine as this is the route for elimination of unejaculated sperm.

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Prostatic disease

- dogs with prostatic disease may have large number of polymorphonuclear neutrophils and bacteria in the ejaculate to which sperm may become attached
- the effect of sperm clumping appears not to have a significant impact upon fertility
- however whether changes in ejaculatory fluid pH or ionic composition have an impact upon fertility is no known
- unfortunately many treatments for prostatic disease require administration of hormonal preparations which themselves adversely affect semen quality

Synthetic glucocorticosteroid therapy

- continual administration of glucocorticoids at high doses has a rapid deleterious effect on semen quality which is probably mediated in the epididymis
- the effect of shorter-duration therapy and lower doses has not been quantified

20.6 IMPOTENCE (INABILITY TO COPULATE)

- many causes of poor libido are described (see 20.2)
- inability to copulate may also occur as a result of a painful condition manifest at the time of mating, including prostatic, penile or skeletal pain; in these cases the dog will appear to be interested in a bitch in oestrus but will not be able to mount, gain intromission or achieve a 'tie'

20.7 ATTRACTIVENESS TO OTHER MALE DOGS

- in juvenile males kept together often the subordinate male will be subjected to mounting by males that are higher in the social hierarchy
- occasionally, however, males become attractive to other males who may demonstrate behavioural responses typical to those presented with a bitch in oestrus
- some of these cases are entire males with a testicular tumour producing oestrogen
- other cases occur in males many years after they have been castrated
- in castrated males no hormonal cause has been identified, but circulating testosterone concentrations are low; it is possible that other adrenal-produced oestrogens are responsible for the condition, although adrenal ultrasound usually does not demonstrate a lesion
- the response of the other males suggests that pheromones are produced
- some control of the problem can be achieved by administration of depot androgens or even depot progestogens, which supports that fact that this is an endocrine abnormality amenable to hormonal suppression

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REFERENCES

1 England, G.C.W. (1997) Effect of progestogens and androgens upon spermatogenesis and steroidogenesis in dogs. *Journal of Reproduction and Fertility* **51**(Supplement), 123–138.

2 England, G.C.W. and Allen, W.E. (1991) The effect of the synthetic androgen mesterolone upon seminal characteristics of dogs. *Journal of Small Animal Practice* **32**, 271–274.

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21 Abnormal Pregnancy

21.1 FERTILIZATION FAILURE

- failure of fertilization is most commonly the result of oocytes and sperm not being present in the female tract together at the same time; this is usually because the owner decides when to breed and may chose an inappropriate mating time (see 11.1)
- fertilization failure may be the result of an inherently poor semen quality
- sperm that have been cryopreserved also have poor quality and a substantially reduced period of survival in the female tract
- otherwise there are few reports of conditions that impact upon fertilization
- it is possible that an inflammatory disease of the uterine tube (called salpingitis) may impact fertilization but there are few reports of this condition
- rarely, anovulation may occur (see 19.4)
- fertilization may be prevented by post-coital administration of oestrogens

21.2 EARLY EMBRYONIC DEATH

- loss of embryos before a diagnosis of pregnancy can be made almost certainly occurs but the rate and time at which this occurs is not known
- most commonly this condition is seen in healthy bitches with cystic endometrial hyperplasia; here an abnormal uterine environment allows bacteria that enter the uterus at oestrus to persist and interfere with normal embryo development
- both pregnancy rate and litter size are reduced in bitches with endometrial hyperplasia compared with normal bitches; endometrial hyperplasia can be diagnosed using diagnostic ultrasound (see 16.5)
- there is limited evidence that administration of systemic antibiotic post mating may help resolve the endometrial inflammation and improve pregnancy rate and litter size [1]
- it is also thought that individual embryos may fail due to genetic abnormalities

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21.3 RESORPTION

- in the author's clinic we have found that resorption of one or more embryos but continuation of the rest of the pregnancy occurs in up to 10% of pregnancies and probably represents abnormal development of the embryo or competition of placental attachment sites (as the condition is more common in large litters) [2]
- resorption of an entire litter may also occur but is usually the result of an infectious agent (these are detailed in 21.5)
- resorption only occurs when an embryo fails before day 35
- embryonic resorption can be detected by ultrasound examination (Figure 21.1)
- ultrasound can also be used to predict resorption since embryos destined to fail usually are slow to develop and grow at a slower rate than normal embryos
- the author's clinic was the first to document with ultrasound normal embryonic growth rates for the dog; these growth curves are now widely used in clinical practice to determine whether embryos are normal or abnormal [3,4]
- resorbing embryos usually have an irregular outline, there may be separation of membranes from the uterine wall, the yolk sac fluid becomes echogenic, there may be inward bulging of the uterine wall, and ultimately the fluid will disappear leaving a heterogenous region (Figure 21.2)
- after embryonic death the fluids are resorbed into the bitch's circulation and the tissues (membranes and embryo/fetus) become dehydrated and autolysed
- bitches should be tested for canine herpes virus when complete resorption occurs; vaccination at the next pregnancy may prevent the problem from occurring again and reduce the risk of abortion and fading puppy syndrome

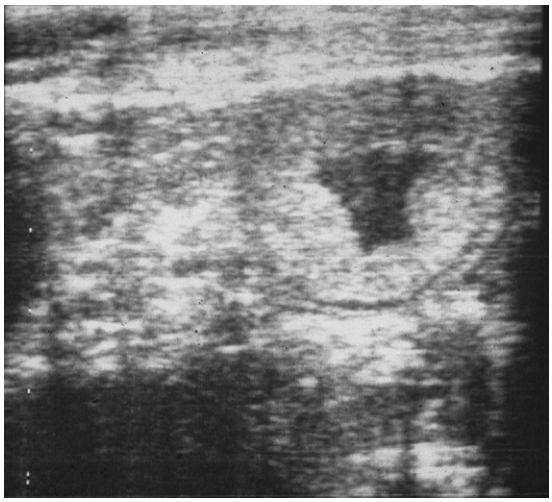


Figure 21.1 Isolated embryonic resorption in a bitch. There is loss of the normal spherical outline, inward bulging of the conceptual wall and increased echogenicity of the yolk sac fluid. Five normal conceptuses were also present and these were delivered normally at term. 7.5 MHz transducer.

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Figure 21.2 Ultrasound image of pregnancy at 20 days; there is an irregular corrugated appearance to the outline of the conceptuses; pregnancy failure by resorption occurred 3 days later.

- with the exception of German shepherd and similar breeds which exhibit ovulation abnormalities and short luteal phase, the author does not believe that resorption is commonly the result of progesterone deficiency
- in breeds where a short luteal phase occurs it may be feasible to monitor progesterone concentrations using an assay with high sensitivity; progesterone concentrations less than 2 ng/ml necessitates treatment with exogenous short-acting progestogens (commonly oral altrenogest at 0.1 mg/kg is used although this author has seen even this low dose cause urogenital abnormalities in developing fetuses and prefers to use 0.05 mg/kg)

21.4 UTERINE BODY PREGNANCY

- occasionally a conceptus develops in the body of the uterus and the placenta becomes closely apposed to the cervix
- after about 30 days of pregnancy, blood from the marginal haematoma starts to leak through the cervix
- bleeding is often of a small volume but can be severe resulting in anaemia in which case medical pregnancy termination or an ovariohysterectomy may be considered
- ascending bacterial infection does not appear to occur
- in a small number of cases the body pregnancy is expelled spontaneously; it is remarkable that the cervix may open, uterine contraction cause expulsion, but the remaining pregnancies are protected
- in bitches with uterine body pregnancies, the viability of the remaining pregnancies should be monitored using real-time ultrasound

21.5 ABORTION

- there is no evidence to suggest that abortion may occur as a result of physiological or minor nutritional stress; bitches must be severely ill before a pregnancy is terminated, or there must be specific bacterial or viral infectious agents

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Bacterial causes of abortion

Brucella canis infection

Brucella is present throughout much of the world and although it is not currently present in some countries, because of the wide international movement of bitches for breeding purposes it must be considered an important differential diagnosis.

- bitches with Brucella often have generalized lymph node enlargement and generally abort after day 35 of pregnancy; some cases suffer earlier embryonic resorption
- aborted material (and semen from males) is highly infectious
- bacteraemia may persist for years and any response to treatment is poor
- affected animals should never be used for breeding
- the focus is best paid to careful screening of all animals before breeding

Miscellaneous bacteria

- sporadic cases of abortion are found associated with Escherichia coli, Campylobacter, Pseudomonas, Salmonella, haemolytic streptococci and clostridia
- occasionally several bitches in a kennels are affected
- it is assumed that these organisms persist in the uterus after mating or enter via the cervix in later pregnancy
- generalized malaise or infectious disease is rarely associated with abortion
- the bitch usually expels all fetuses and membranes
- the fetal stomach is the best place to sample for bacteriology; bacteria found here were ingested in utero
- broad-spectrum antibiotic treatment for aborting bitches is prudent until results of bacteriology are known
- Brucella abortus has been known to produce abortion via infected cows' milk, but this is now unlikely in many countries

Viral causes of abortion

Canine herpes virus

- in adult dogs canine herpes virus generally produces a few mild signs limited to the respiratory or genital tract
- the uncomplicated disease may be unnoticed by the owner
- genital tract lesions are frequently raised nodules that are red in colour; these are present within the vestibule and vagina of the bitch and also within the prepuce of the dog
- in the bitch lesions are evident at the onset of pro-oestrus, suggesting venereal transmission is probably important
- recrudescent virus shedding from the vesicular lesions may be stimulated by the stress of pregnancy and parturition

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- infection in the bitch may be associated with infertility, abortion and stillbirths
- infection of the pregnant bitch results in placental lesions and the infection of the fetuses:
  (a) infection during early pregnancy may result in resorption or fetal death and mummification
  (b) infection during mid pregnancy results in abortion
  (c) infection during late pregnancy results in premature birth
- pups may become infected at birth, during passage through the vagina, and subsequently die with widespread necrotizing lesions
- a small number of pups may survive such infection and in these signs of cerebellar and retinal dysplasia have been observed, and in one case a late-onset (at 3 years of age) cerebellar degeneration has been reported
- pathological changes in pups are often characteristic of canine herpes virus infection, and virus can be isolated readily from these tissues
- infection in the adult dog often requires no treatment and the condition is self-limiting
- in pregnant bitches supportive therapy during the abortion is required
- in the pups, the disease is rapidly fatal and treatment is often unrewarding
- bitches which have previously given birth to infected pups may deliver another infected litter but later may deliver a normal litter
- resorbing and aborting bitches should be isolated from other pregnant bitches
- infected bedding should be removed and the environment disinfected
- the initial source of the virus is oral, nasal or vaginal secretions (and possibly preputial excretions)
- the potential for viral transmission at coitus is uncertain; however, males with reproductive tract lesions should not be used for mating
- vaccination against canine herpes virus is possible; the first vaccination is given during oestrus or within the first 10 days after mating and a second vaccination is given at approximately 55 days of pregnancy; high success rates of preventing pregnancy loss and fading pups have been achieved, re-vaccination is required for each pregnancy

Canine distemper virus

- in susceptible dogs a peracute form with sudden onset pyrexia and death may occur
- most infections result in primary respiratory tract or gastrointestinal tract signs
- some dogs may develop central nervous system lesions
- some may develop hyperkeratosis of the pads and nose ('hardpad')
- experimental exposure of pregnant bitches produces either:
  (a) clinical illness in the bitch with abortion, or
  (b) subclinical infection of the bitch and the birth of clinically affected pups
- diagnosis is via postmortem, histopathology and virus isolation
- there is no suitable treatment for the reproductive tract consequences of distemper virus infection

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- vaccination is now performed using attenuated live virus vaccines as part of annual or biennial programmes

Canine parvovirus

- canine parvovirus 1 has not been confirmed as a major cause of disease
- canine parvovirus 2 appears to have arisen as a host range mutant of feline parvovirus
- the virus may cross the placenta and affect pups which then develop acute generalized infection and myocardial disease soon after birth
- a diagnosis may be made upon the clinical signs, supported by a leucopenia, and the identification of virus or viral proteins
- the reproductive consequences can be treated by supportive nursing of affected pups, especially using fluid therapy and antimicrobial agents
- inactivated canine parvovirus vaccines are available, and recently live tissue culture attenuated vaccines have been selected and are widely used as part of annual vaccination programmes

Canine adenovirus

- canine adenovirus 1 is a highly contagious disease known to produce infectious canine hepatitis
- the virus may cross the placenta during late pregnancy, resulting in the birth of pups which rapidly develop signs of systemic infection similar to those observed in older pups and adults
- some pups may be born dead whilst others are weak and die within a few days of parturition
- infection may be diagnosed upon the basis of the clinical signs, and virus isolation
- adjuvanted inactivated vaccines are available and in certain cases their single administration can provide immunity for life

Protozoal causes of abortion

Toxoplasma gondii

- this has rarely been implicated in canine abortion; the cat is the definitive host
- infection may occur following ingestion of infected meat or the faeces of an infected cat
- it seems likely that pups are only affected if ingestion and the acute phase of infection occurs during pregnancy and there is transplacental infection (chronic infection in the bitch is not passed to her fetuses)
- if abortion does not occur in the acute phase of infection it is possible that pups will be born with neurological signs
- control is relatively simple by preventing ingestion of infected cysts by the pregnant bitch

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Abortion of unknown aetiology

- bitches with uterine disease may suffer embryonic resorption and it is feasible that fetal abortion also occurs; uterine disease can be detected by ultrasound examination and this is best performed during the luteal phase when progesterone concentrations are high
- there is no clear data to show that low plasma progesterone concentrations cause resorption or abortion except in German shepherd and similar breeds where an anovulation/short luteal phase syndrome has been reported
- ingestion of toxic substances is rarely identified as a cause of abortion
- although there are many potentially teratogenic substances, they are rarely a cause of pregnancy failure in bitches

Partial abortion

- there are several recorded cases of bitches aborting some pups and having others normally at term; this appears most commonly in cases with a uterine body pregnancy (see 21.4) [5]
- the presence of live pups can be confirmed by ultrasound examination
- antibiotic therapy should be given after abortion to try to prevent ascending bacterial infection affecting the other foetuses
- it is inappropriate to consider the administration of progesterone or progestogens without measuring plasma progesterone concentrations; if supplementation is considered this should be a short-acting oral agent administered daily and therapy should be ceased 4 days before expected parturition otherwise parturition will be prevented

NB: Tetracycline antibiotics should not be given to pregnant bitches as they may cause discoloration of the pups' teeth. Ampicillin and amoxicillin are the safest antibiotics to use during pregnancy.

Prevention of abortion

- in bitches with a history of abortion, the following may be tried:
  (a) broad-spectrum antibiosis immediately after mating to reduce bacterial colonization of the uterus
  (b) broad-spectrum antibiosis (not tetracyclines) at the time of expected abortion in the belief that bacteria enter the uterus via the cervix during pregnancy
  (c) progesterone supplementation in the belief that low progesterone concentrations result in abortion. This has never been proven except for German shepherds and similar breeds and following oestrus-induction regimes. Administration of progesterone or progestogens during pregnancy may delay or prevent parturition, and may cause urogenital abnormalities in the pups

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21.6 FETAL ABNORMALITIES

- a variety of fetal abnormalities may be present in utero
- some of these are not compatible with survival and result in abortion
- fetal normality and abnormality has been documented using real-time diagnostic ultrasound (see 13.9)
- the author's clinic was the first to detail normal fetal growth rates and detection of fetal abnormalities; data which are now in wide clinical use [3,6,7]

21.7 METROCOELE

- rarely a loop of uterus can protrude through the inguinal ring and be positioned under the skin in the inguinal region
- if the bitch becomes pregnant a swelling (conceptus) may start to develop in this area
- early detection of the problem may allow the uterus to be returned to the abdomen surgically; if this is not noticed the uterus may be strangulated, even if not parturition is impossible
- most commonly late detection occurs and hysterotomy or hysterectomy must be performed

21.8 UTERINE RUPTURE

- most cases can be traced back to some obvious abdominal trauma during pregnancy
- cases may however be asymptomatic at the time
- the pups which are expelled from the uterus die and become dehydrated (mummified); even at this stage if there is no bacterial contamination there are no clinical signs
- mummified pups are sometimes discovered during routine examination or laparotomy
- the condition may also occur during parturition (see 22.9)

21.9 LOSS OF FETAL FLUID

- normally fetal fluid is only lost after active signs of parturition have commenced
- however in in late pregnancy this occasionally occurs spontaneously when the bitch is suffering a hydrops condition (it is unknown whether this is hydrops amnii or hydroallantois)
- release of fluid does indicate that the cervix is open and the fetal membranes have ruptured so this sign must be taken seriously as the pregnancy is threatened

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21.10 PREGNANCY DIABETES

- increased concentrations of progesterone during pregnancy (and non pregnancy) causes increased concentrations of growth hormone which can have an insulin antagonistic effect resulting in diabetes mellitus
- clinical signs are polyphagia and polydipsia/polyuria, usually with a surprising (to the owner) weight loss
- this condition is reversible once progesterone concentrations decrease and so treatment is usually not required
- in cases with severe clinical signs medical termination of pregnancy may be necessary (see 27.8 and 27.9)

21.11 HYPOGLYCAEMIA

- this condition is rare during pregnancy because elevated concentrations of progesterone result indirectly in peripheral insulin antagonism (see pregnancy diabetes above)
- however a primary hypoglycaemia has been reported in pregnancy which appears to disappear after parturition
- the aetiology is unknown but clinical signs are typical of hypoglycaemia: weakness, collapse and ultimately coma
- diagnosis can be made by measurement of plasma glucose concentrations and treatment with intravenous glucose produces a rapid clinical response

REFERENCES

1 England, G.C.W., Moxon, R. and Freeman, S.L. (2012) Delayed uterine fluid clearance and reduced uterine perfusion in bitches with endometrial hyperplasia and clinical management with post-mating antibiotic. *Therigenology* (in press).
2 England, G.C.W. (1992) Ultrasound evaluation of pregnancy and spontaneous embryonic resorption in the bitch. *Journal of Small Animal Practice* **33**, 430–436.
3 England, G.C.W., Allen, W.E. and Porter, D.J. (1990) Studies on canine pregnancy using B-mode ultrasound; development of the conceptus and determination of gestational age. *Journal of Small Animal Practice* **31**, 324–329.
4 England, G.C.W. and Russo, M. (2006) Ultrasonographic characteristics of early pregnancy failure in bitches. *Theriogenology* **66**, 1694–1698.
5 England, G.C.W. (1998) Ultrasonographic assessment of abnormal pregnancy. *Veterinary Clinics of North America: Small Animal Practice* **28**, 849–868.
6 Allen, W.E., England, G.C.W. and White, K.B. (1989) Hydrops foetalis diagnosed by real time ultrasonography in a bichon frise bitch. *Journal of Small Animal Practice* **30**, 465–467.
7 England, G.C.W. and Allen, W.E. (1990) Studies on canine pregnancy using B-mode ultrasound; Diagnosis of early pregnancy and the number of conceptuses. *Journal of Small Animal Practice* **31**, 321–323.

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22 Abnormal Parturition and Problems After Whelping

A normal birth is termed 'eutocia', whilst the word 'dystocia' is used to describe any problem which interferes with normal birth.

22.1 CLASSIFICATION OF DYSTOCIA

Difficult birth is often described as being associated with obstruction or poor uterine contractions.

Obstructive dystocia

- this may occur because of problems of the dam or the fetus

Maternal dystocia

- caused by, for example, a narrow birth canal caused by a pelvic fracture or a vaginal stricture

Fetal dystocia

- caused by for example, a large pup or malpositioned or abnormal pup

Uterine inertia (poor uterine contractions)

- this may occur as a primary problem or secondarily following an obstructive dystocia

Primary uterine inertia

- absence of uterine contractions and therefore absence of first stage parturition and no progression to second stage parturition
- these cases seem to be associated with low plasma oxytocin concentrations

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- the cervix relaxes but pups do not enter the vagina and there is no external sign of parturition – if untreated the pups die
- the cause is unknown but may be due to:
  (a) a small number of pups (one or two) producing an inadequate stimulus to initiate complete parturition
  (b) a very large litter (or excess fetal fluid – 'hydrops') causing overstretching of the uterus and preventing myometrial contractions
  (c) insufficient calcium (subclinical eclampsia) in the myometrium; this may be related to a dietary insufficiency, inadequate parathyroid hormone or a refractory parathyroid gland
  (d) unknown but inherited causes

Secondary uterine inertia

- cessation of uterine contractions after they have started
- presumably due to exhaustion of the myometrium or some of the factors involved in primary inertia
  (a) secondary inertia early in second stage parturition usually follows an obstructive parturition dystocia (unproductive straining)
  (b) secondary inertia later in second stage parturition may occur apparently spontaneously after the normal birth of several pups. The length of time for which uterine inactivity can be considered normal is impossible to define; however, if treatment results in the birth of a dead pup, secondary inertia can be retrospectively diagnosed

22.2 THE 'OVERDUE' BITCH (see 14.5)

- it is important to remember that pregnancy length from the day of ovulation is very tightly regulated in the bitch (65 +/– 1 day) but that mating both before and after ovulation can achieve pregnancy and result in pregnancy lengths that appear longer or shorter than expected respectively
- when the interval from mating exceeds 68 days there is often concern that parturition is overdue; but pregnancy can be 72 days from mating to the onset of parturition and still be normal
- possible causes for the bitches that are thought to be overdue include:
  (a) early mating (see 12.1) in relation to the time of ovulation
  (b) inaccurate information or calculation of expected whelping day
  (c) non-pregnancy
  (d) pregnancy loss, e.g. resorption or abortion
- for investigation of these cases, the mating date should be checked, pregnancy should be confirmed by abdominal palpation, ultrasound and/or radiography used to examine the pregnancy, blood progesterone concentration should be measured, and an endoscopic or digital examination performed

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Examination of the presumed overdue bitch

Mating dates

- examination of the breeding records will enable confirmation of the possible dates of parturition
- parturition may occur as early as 56 days or as late as 72 days after mating
- however if mating was based on determination of the time of ovulation (for example by measurement of progesterone [see Chapter 11]), parturition can be expected 63 (+/– 1 day later)

Abdominal palpation

- pregnancy may be confirmed by palpation of fetuses in late pregnancy; this often gives little information about the time of expected parturition rather simply confirms pregnancy

Clinical examination

- the clinical condition of the bitch is important and may influence intervention decisions later especially if she is debilitated
- presence of milk in the mammary glands is unhelpful as a clinical sign as this may also occur in pseudopregnancy

Ultrasound examination

- ultrasonography will confirm pregnancy and the viability of those pups which are accessible to the ultrasound beam
- ultrasonographic signs of fetal death include:
  (a) absence of fetal heart beat (Figure 22.1)
  (b) absence of fetal movements

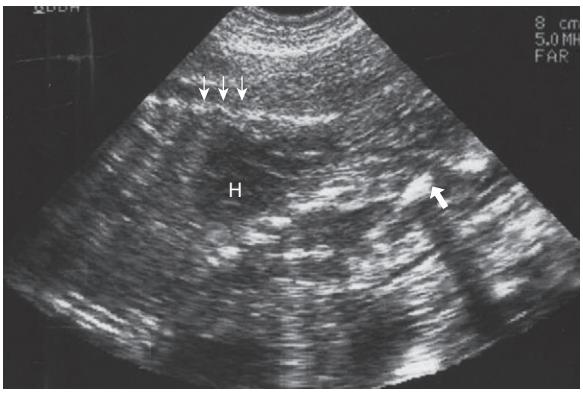


Figure 22.1 Ultrasound image of a dead fetus *in utero*. The fetal ribs (small arrows) and scapulae (arrow heads) can be seen; however the fetal heart (H) has stopped moving and no longer appears anechoic. 5.0 MHz transducer, scale in centimetres.

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(c) reduced volume of fetal fluid
(d) accumulation of gas in the fetal stomach or subcutaneously

- ultrasound examination should be used to confirm fetal viability by counting heart rates (which should be less than twice maternal); a reduction in fetal heart rate is a common clinical sign that the pups are hypoxic

Radiographic examination

- radiography can be used to count the number of fetuses; indeed in late pregnancy this is more accurate than ultrasound examination
- radiography can also be used to confirm fetal death by noting:
  (a) overlapping of the skull bones
  (b) accumulation of gas in the fetal stomach or subcutaneously
  (c) abnormal fetal posture
  (d) additionally, fetal bones in the bitch's stomach may indicate that she has whelped/aborted and eaten the pups
- however radiography is less sensitive than ultrasound for detecting fetal death; it takes several days after death before these signs become apparent

Measurement of plasma progesterone

- progesterone can be easily measured by ELISA in the practice laboratory and results obtained within 45 minutes
- in the author's clinic we have shown that plasma progesterone concentrations decrease 24–36 hours before parturition (Figure 22.2) [1]:
  (a) detection of high progesterone concentrations indicates that parturition is not imminent

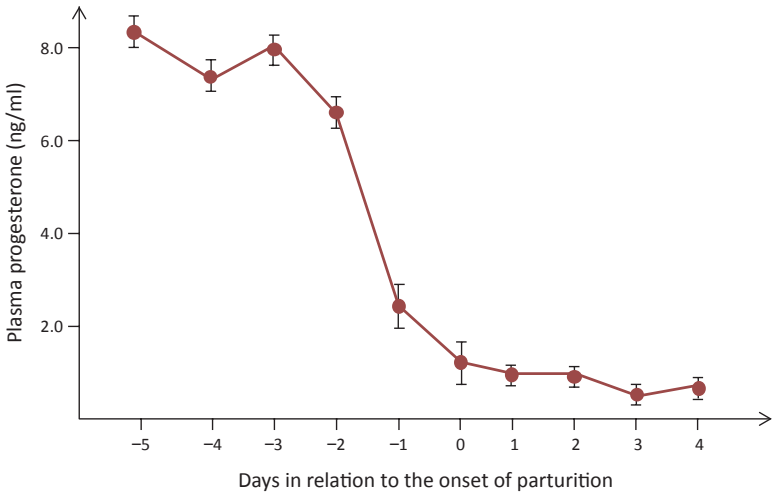


Figure 22.2 Periparturient changes in plasma progesterone concentration.

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(b) basal progesterone concentrations indicate that parturition should have begun
(c) intermediate progesterone concentration are difficult to interpret

Milk progesterone and ionic composition of milk

- in some species changes in progesterone in milk and increasing concentrations of calcium can be used to predict the onset of parturition
- in the author's clinic we have shown that, although similar trends are present in the bitch, the individual animal variation was too large to prevent this from being clinically useful [2]

Digital or endoscopic examination

- it is not possible to palpate the cervix digitally; however, endoscopic examination may allow inspection of the cervix and cranial vagina
  (a) the cervix will be dilated and fetal membranes protrude once second stage parturition has commenced

Treatment options for the presumed overdue bitch

- a number of treatment options are available depending upon the diagnosis reached:
  (a) non-pregnant animals need no treatment
  (b) if the bitch is not at term re-examination should be performed a few days later
  (c) if primary inertia is suspected due to temperature fall (see 14.4) more than 48 hours previously, or evidence of placental separation (see 22.3), oxytocin may be tried but caesarean operation is more successful
  (d) if dead pups are identified, caesarean operation (oxytocin may be tried first, but is unlikely to work)

22.3 BLACK/GREEN DISCHARGE WITH NO SIGNS OF PARTURITION

- a haemorrhagic black/green discharge occurs when fluid escapes from the marginal haematoma of the placenta; this finding therefore indicates that placental separation has begun
- there are a variety of possible causes;
  (a) primary uterine inertia with fetal (at least one) death and placental separation: this may be in a bitch known to be pregnant, or often in an aged bitch which was not known to have been mated and has only conceived one pup

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(b) death of all the fetuses in mid to late pregnancy followed by haematic mummification; the cause is unknown and the pups may die at different stages of development

- ultrasonography is the diagnostic method of choice; importantly counting the fetal heart rate will provide useful information since in the normal fetus this is more than twice or three times the maternal heart rate, a bradycardia indicates fetal distress (essentially the normal chronic response of the fetus to hypoxia is bradycardia)
- radiology may confirm fetal death but only several days after it has occurred
- these cases are invariably treated by caesarean operation

22.4 NERVOUS INHIBITION OF PARTURITION

- in this case the bitch may be in first or second stage parturition but will not settle to whelp properly and may have successive pups in different places, or carry them about
- it is proposed that these occur when the bitch is acclimatized to the whelping box too late or is put into an unfamiliar place to whelp (e.g. the garage)
- ultimately it may be best to relocate the bitch and she will settle and whelping will continue
- the condition can be prevented by allowing the bitch to whelp where she wants, ensuring that the owner is present until whelping is over, and preventing unfamiliar persons from attending the whelping

22.5 INEFFECTUAL STRAINING FOR OVER 1 HOUR

- it is important to recognize the differences between uterine contractions (see during first stage parturition by careful observation of the bitch's flank) and the second stage contractions, which are manifest by active abdominal pushing
- in the normal bitch there are clear signs of progression of parturition within 1 hour from the expulsion of fluid and at least part of a fetus is presented within the birth canal
- whilst the first pup may live for 6 hours after the onset of straining; subsequent pups usually die if they are not delivered after 2 hours of straining
- ineffective straining with no progression usually indicates that the first pup has not entered the bony pelvis/vagina, is slow to progress through the vagina, or is obstructed within the vagina

Common presentations

Pup not entering the birth canal

- may be associated with:
  (a) large pup (especially in small litter)
  (b) fetal monster, e.g. hydrocephalus, anasarca

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(c) transverse presentation, i.e. pup partly in both uterine horns; it cannot then enter the uterine body
(d) ventral flexion of the nose so that the poll of the head engages the pelvic inlet
(e) lateral deviation of the head and neck; may feel one leg in vagina
(f) complete ventral deviation of head and neck
(g) pendulous abdomen where the pup cannot get up to pelvic inlet
(h) dead pup
(i) maternal pelvic abnormality or rarely a uterine torsion

Pup within the vagina, but progressing slowly

- may be associated with:
  (a) relatively large pup
  (b) posterior presentation of first pup
  (c) straining not intense enough

Pup obstructed within the vagina

- may be associated with:
  (a) tumour (polyp)
  (b) exostosis or pelvic bone misalignment following a previous fracture
  (c) congenital vaginal stenosis, usually due to a longitudinal band of tissue which runs the length of the vagina; presumably such bitches become pregnant without a 'tie' (see 9.4 and 9.5)

Clinical approach

- in all of these cases the mainstay of diagnosis will be careful digital examination (remember it is not possible to palpate the cranial vagina even in a small-sized bitch) and ultrasound examination to confirm fetal viability
- possible findings from digital exploration of the vagina include:
  (a) vagina relaxed and moist but no pup present; manipulating a pup in the caudal abdomen with the other hand may make palpation easier
  (b) fluid-filled amnion in vagina
  (c) part of a pup in the vagina
  (d) vaginal occlusion due to a polyp, exostosis or stenosis
- treatment options include:
  (a) if nothing felt in vagina – caesarean operation
  (b) if amnion only felt – lift fetus transabdominally with other hand and stimulate the dorsal wall of the vagina to cause contractions
  (c) if vaginal occlusion – caesarean operation
  (d) if fetal malposition – see Obstetrical manipulation (22.8)

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22.6 BITCH HAD SEVERAL PUPPIES BUT RESTLESS OR LARGER LITTER EXPECTED

- a variety of causes are seen of which secondary uterine inertia is the most likely (exhaustion of the myometrium)
- it is possible that this is the normal rest between deliveries which can be very long at the end of parturition especially if this is a large litter
- it is possible that all pups have been delivered
- these cases should be investigated by digital exploration per vaginam and ultrasound examination of the uterus
- cases of secondary inertia will reveal undelivered pups which may or may not be viable
- as long as no obstruction is present oxytocin can be administered and the bitch should be carefully observed for response to treatment
- if there are signs of fetal stress (documented by reduced heart rate) or dead fetuses are present then caesarean operation should be performed

22.7 OXYTOCIN TREATMENT

- oxytocin causes rhythmic uterine contractions as long as it is given at the time of parturition
- oxytocin has only a pharmacological effect if given more than 36 hours after parturition
- its half-life is short, but it can cause powerful contractions
- small doses, e.g. 2–4 IU, should be administered initially; if no effect is seen after 10–20 minutes, the same or a larger dose may be given; it may be appropriate to consider calcium administration at the same time as the uterus responds poorly if the bitch is hypocalcaemic
- if delivery of a pup is stimulated by oxytocin, administration may be necessary for subsequent pups
- where many pups are still to be born, the use of caesarean operation should be considered because although oxytocin may be effective it is likely that the duration of parturition will be long and there is risk to the last pups
- oxytocin should not be given in obstructive dystocia or if the cervix is not thought to be relaxed (this requires endoscopic inspection to accurately document)

22.8 OBSTETRICAL MANIPULATION

- strict attention should be paid to aseptic preparation before undertaking any examination
- drying of the vagina should be avoided by using a water-soluble lubricant
- it can be helpful to confirm that a pup is alive; this can be achieved because most live pups make sucking movements when a finger is put in their mouths,

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it may be possible to palpate a pulse or heart beat, or in some there may be a withdrawal reflex when the toes are squeezed
- in most cases repositioning of the pup is best achieved with the pup retropulsed into the birth canal, after correction of alignment traction may then be provided:
  (a) by finger manipulations and by pushing the pup caudally using transabdominal manipulation with the other hand
  (b) the pup's head or hips may be gripped between the crooked index and second fingers
  (c) flexed front legs and back legs (breech) may be reached by passing the index finger transversely over the dorsum (shoulder or pelvis) of the pup and flicking the limb backwards; the procedure is repeated with the other hand in bilateral flexions
  (d) a vectis forceps or a strong teaspoon may be used like a vectis; this cups the dome of the head and is not likely to cause significant trauma
  (e) using whelping forceps, however these must be used with care to ensure the vagina is not snared, the fetus is not inadvertently crushed or fetal extremities are not amputated
- if a pup appears to be 'stuck', slight rotation may assist delivery
- traction should be applied if possible whilst the bitch is straining
- pressure on the vaginal roof with the fingers may stimulate straining
- caesarean operation should be considered if:
  (a) no progress is made after 15 minutes
  (b) the vagina becomes traumatized
  (c) the bitch becomes distressed
  (d) there are many pups yet to be born

NB: The decision to use caesarean operation is often difficult at the time and retrospective analysis is always easy; the responsibility of the clinician is for the comfort and life of the bitch and her pups. It is often quicker and safer to perform a caesarean operation than to embark on obstetrical manipulation, particularly in small bitches; relief of obstructive dystocia is often followed by secondary inertia.

22.9 UTERINE RUPTURE

- this appears to occur in mid pregnancy (see 21.8) and also appears to develop spontaneously at term
- pups are found alive or dead in the abdomen at laparotomy; rupture of the uterus is evident
- the bitch may be systemically unwell with signs of polydipsia, vomiting, depression and pyrexia
- diagnosis before surgery is often difficult
- fetuses which have been in the abdomen for some time will be mummified and may remain there for years
- extensive abdominal adhesions may result

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22.10 UTERINE TORSION

The published literature shows more bitches with uterine torsion were not pregnant rather than pregnant.

- in pregnant bitches the condition usually occurs in late pregnancy and most commonly affects one horn
- if there is vascular compromise the condition causes severe abdominal pain and reluctance to move
- can only be diagnosed at laparotomy
- hysterectomy or hysterotomy may be performed depending upon the viability of the uterus

22.11 RETAINED FETAL MEMBRANES

- the condition is uncommon
- the placenta is usually but not always passed within 20 minutes of each pup; sometimes two or more pups are delivered before the placentas are expulsed
- in many cases retention is suspected because not all placentas have been seen by the owner, although two or more may have been delivered together, or may have been eaten by the bitch
- retention is usually suspected if a green/black discharge persists after parturition
- diagnosis is difficult and requires ultrasound examination or clinical experience
- in suspected cases, broad-spectrum antibiotics should be given
- oxytocin has little effect after parturition because oxytocin receptors rapidly disappear; low-dose prostaglandin treatment may be effective
- metritis is a common complication

22.12 POST-PARTUM METRITIS

- metritis arises because of bacterial invasion of the uterus following parturition, abortion, manipulation or placental retention
- there is normally a foul smelling red-coloured discharge from the vulva
- the bitch becomes pyrexic, toxaemic, depressed, anorexic and milk production decreases
- pups receive little milk, may not be nursed by the dam and often cry because of cold and hunger
- the condition may be fatal despite treatment which in the first instance is directed to control of the bacterial infection (e.g. by the use of intravenous ampicillin or potentiated sulphonamide), replacing fluid loss (e.g. Hartmann's solution) and encouraging uterine evacuation (e.g. administration of oxytocin or prostaglandin)

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- it is best to collect a guarded swab from the cranial vagina so that, on the basis of culture and sensitivity, the antibiotic can be changed retrospectively
- ovariohysterectomy may be considered if a pup is retained
- uterine lavage with very dilute chlorhexidine via laparotomy has been described but the uterus is very friable and may not be amenable to such an approach

22.13 POST-PARTUM HAEMORRHAGE

- a small volume of haemorrhagic discharge is common at parturition
- larger volumes of blood loss usually originate from uterus endometrial oozing but may also be the result of physical injuries to the vagina
- in these cases flesh blood is passed which clots
- if a vaginal lesion is recognized a tampon may be useful to apply direct pressure to the site, otherwise an assumption is made that the bleeding originates from the uterus and oxytocin and antibiotics are administered; uterine contractions result in an initial expulsion of blood and make the condition look worse, ergometrine may be preferable to oxytocin because it provides a prolonged initial contraction of the uterus
- in bitches that do not respond to treatment a coagulopathy should be considered
- blood transfusion and/or ovariohysterectomy may be considered

22.14 SUBINVOLUTION OF PLACENTAL SITES

- a haemorrhagic discharge is common for 2 weeks after normal parturition, and a mucoid discharge will often be present for a few more weeks
- if a serosanguinous discharge persists, it is most probably due to subinvolution of the uterus at one or more of the placental sites; haemorrhage continues from the endometrium at the position of the marginal haematoma (see 12.4)
- these bitch show no signs of malaise, and dilated regions of the uterus can be detected using ultrasound imaging
- antibiotics and ecbolic drugs do not help to terminate the condition which generally persists but with declining blood loss until there is re-vascularization at the subsequent oestrus
- in one study a combination of progestogens and antibiotics was found to be useful in a limited number of cases
- subsequent fertility is not affected
- if bleeding is marked, the bitch should be examined for anaemia, which, if present, may necessitate an ovariohysterectomy

22.15 HYPOCALCAEMIA (PUERPERAL TETANY OR ECLAMPSIA)

Although large studies have not been conducted it appears that hypocalcaemia is most common in small breeds and is seen in early lactation or less commonly in late pregnancy.

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- clinical signs are nervousness, panting, whining, hypersalivation and a stiff gait
- there is frequently a marked pyrexia
- if untreated, tonic or clonic muscular spasms may occur
- treatment is relatively simple by slow intravenous administration of 5–20 ml 10% calcium borogluconate; cardiac rate and rhythm should be monitored during intravenous administration
- the same volume of calcium should be administered subcutaneously and some bitches need continued supplementation through lactation, simply given using human antacid tablets
- it is best to try to feed the pups artificially for 24 hours after the acute episode or to wean completely; if they are weaned completely a prolactin inhibitor (cabergoline) can be given to stop lactation
- oral calcium supplementation may be tried in bitches at risk (those that have previously had the problem) but this may depress parathyroid hormone production and exacerbate the condition

22.16 CANNIBALISM

- nervous (often inexperienced) bitches may neglect or kill pups if they are disturbed, particularly by strangers
- bitches may neglect individual pups; sometimes they have obvious defects, e.g. small size, cleft palate
- excess licking may cause ulceration of the limbs etc. of pups

22.17 PROLAPSED UTERUS

- this condition is very rare and only occurs during the periparturient period when the cervix is open
- it may be complete or incomplete in which case the bitch strains and the prolapse can be palpated in the vagina
- in cases of complete prolapse fatal haemorrhage may occur from the uterine vessels
- laparotomy may be used to pull the uterus back into the abdomen

22.18 FAILURE OF MILK LET-DOWN

- failure of milk let-down is more common than agalactia and is usually the result of adrenalin release (due to stress or pain, for example, a caesarean operation) which inhibits the oxytocin response
- some cases may be associated with physical obstruction of the mammary collecting system because of swelling of the gland
- both forms respond reasonably well to the repeated administration of exogenous oxytocin (2 IU/bitch up to four times daily)
- these cases must be differentiated from agalactia

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22.19 AGALACTIA

- agalactia is an absence of milk; essentially a failure of milk production
- this can be temporary when milk production lags a few days behind parturition, or persistent when milk production is inadequate or inadequate for the size of the litter
- whilst milk production can be stimulated by administration of metoclopramide (1 mg/kg four times daily) the most important issue is supplementary feeding of the pups
- encouraging the pups to suck even if there is no milk will encourage milk production
- these cases must be differentiated from failure of milk let-down

22.20 MASTITIS

- mastitis in the bitch is most commonly seen as an acute inflammatory condition which may or may not have a bacterial aetiology

Types of mastitis

Commonly simply classified as acute or chronic.

Acute mastitis

- most cases are acute bacterial mastitis and are seen within the period of lactation or shortly afterwards
- bacteria isolated are frequently commensal or environmental organisms which seem to be introduced by trauma at sucking or associated with a dirty environment
- the mammary gland is usually hot, swollen and painful (Figure 22.3), and the milk is discoloured red or brown
- the bitch may have just local signs or develop systemic signs and in these latter cases the changes in the milk may affect the pups (toxin milk syndrome)
- some of these cases develop mammary abscesses; the gland may then become cold and ulcerated (gangrenous mastitis)

Chronic mastitis

- cases of subclinical mastitis are believed by some workers to be associated with poor milk quality and failure of pups to thrive
- presumably chronic bacterial contamination of specific lobes occurs in these cases

Treatment of mastitis

- mastitis is usually treated symptomatically with broad-spectrum systemic antibiotics and non-steroidal anti-inflammatory drugs

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Figure 22.3 Acute bacterial mastitis; the gland is hot, painful, swollen and discoloured.

- whilst antibiotic penetration is usually good when the mammary gland is inflamed, levels of antibiotic can be improved by considering the milk pH: when pH is less than 7.2 potentiated sulphonamide is a good choice; when pH is more than 7.4 penicillins are a good choice
- massage of the mammary gland with warm water and stripping of the milk are important adjuvant treatments
- in cases of mammary abscess, surgical drainage may be required

22.21 GALACTOSTASIS

- galactostasis is a build up of large amounts of milk within the mammary gland
- it is seen most commonly in bitches immediately after parturition before pups are activiely sucking from all teats, in bitches that produce more milk than is required, in bitches that are pseudopregnant, and in bitches after weaning
- in most cases the gland is swollen and warm but is not hot and painful, however the condition may progress to mastitis
- in the short term stripping of the gland helps relieve the clinical signs but perpetuates lactation
- cold compresses and the use of prolactin inhibitors such as cabergoline are required to reduce milk production

REFERENCES

1 England, G.C.W. and Verstegen, J.P. (1996) Prediction of parturition in the bitch using semi-quantitative ELISA measurement of plasma progesterone concentration. *Veterinary Record* **139**, 496–497.

2 England, G.C.W. and Verstegen, J.P. (1997) Progestogen concentration and ionic composition of the mammary secretion of periparturient bitches. *Journal of Reproduction and Fertility* **51**(Supplement), 209–214.

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23 Care of the Sick Neonate

23.1 IMPORTANT CONSIDERATIONS

- a number of factors can help reduce sickness in neonates including:
  (a) maintenance of breeding records including details of the health status of breeding stock
  (b) good management during whelping and the immediate postnatal period
  (c) ensuring pups receive adequate colostrum
  (d) recognizing the signs of ill health early
  (e) sending dead puppies for postmortem examination

23.2 SIGNS THAT THE NEONATE MAY BE SICK

- it is important to look for early signs of illness because once illness is established pups can be difficult to treat and mortality is high
- a variety of typical clinical signs that are characteristic of illness include:
  (a) being underweight with a thin, bony appearance and a slack abdomen
  (b) failure to suck
  (c) failing to gain weight at the normal rate or at the same rate as others in the litter
  (d) having cold and inelastic skin
  (e) a coat in poor condition
  (f) persistent crying and possibly a loss of body twitching
- a useful clinical assessment tool has been developed from the 'Agpar' score used in human neonatology; the parameters that comprise the score include heart rate, respiratory effort, mucous membrane colour, muscle tone and response to stimulation (Table 23.1)
- there is good correlation between the Agpar score and the likelihood of survival
- other tests include detailed ultrasound examination of the pups' organ systems; these have been described in pups examined at the authors clinic [1]

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Table 23.1 Scores awarded for various parameters when assessing neonatal viability; the Agpar score is summed for each criteria leading to total scores between 6 (moribund) and 18 (normal).

Criteria evaluated	Score 1	Score 2	Score 3
Heart rate	Absent	Less than 180	More than 180
Respiratory rate	Absent	Increased effort	Normal
Mucous membrane colour	White or bluish	Pale pink	Pink
Muscle tone and motor activity	Absent	Tone but no activity	Movement
Suck reflex	Absent	Weak	Strong
Response to vigorously rubbing back	No response	Moves but no crying	Movement and crying

23.3 TREATING A SICK PUP WITH MINOR SIGNS OF DISEASE

- even very minor clinical signs should initiate implementation of immediate treatment to prevent progression of the disease
- early supplemental feeding is the mainstay of early treatment
- an adequate volume of colostrum or milk should be given every 2–3 hours by feeding bottle or orogastric tube (see 15.4)

NB: The perineal region of each pup should be regularly stimulated to ensure expulsion of urine and faeces.

- body temperature needs to be maintained and should be recorded every few hours along with environmental temperature
- hydration status should be checked and can be estimated from:
  (a) amount of weight gain (measured three times daily)
  (b) mucous membrane colour and skin elasticity (5–7% dehydration – membranes are dry and tacky; 10% dehydrated – membranes are very dry and the skin shows reduced elasticity)
  (c) urine colour (normal hydration presents as urine with no colour)
- pups with minor signs of illness are at risk of overgrowth of commensal bacteria in the gut therefore collection of bacteriological samples from faeces and blood for aerobic and anaerobic culture should be considered
- administration of broad-spectrum antimicrobial preparations (oral clavulanic acid potentiated amoxicillin at 12.5–25.0 mg/kg twice daily) may be considered

23.4 TREATING A SICK PUP WITH SERIOUS SIGNS OF DISEASE

- once pups stop sucking they enter a downward spiral of dehydration, hypothermia, bacterial overgrowth, ileus and circulatory collapse, and death can quickly follow

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Figure 23.1 Pups that have serious signs of disease are best kept in a controlled environment incubator.

- some of these clinical signs complicate the condition and treatment (e.g. ileus results in a bloated abdomen and reduces sucking further)
- clinical signs that demonstrate a progressive decline in the pup include dehydration, persistent crying and failure to feed
- immediate aggressive treatment aimed to reverse (1) hypoglycaemia, (2) hypothermia and (3) hypoxia, and to treat or prevent (4) dehydration and (5) commensal organism overgrowth is required
- throughout treatment intensive monitoring is required and should include:
  (a) assessment of mucous membrane colour and capillary refill time
  (b) measurements of rectal temperature
  (c) monitoring of respiratory and heart rate
  (d) examination of urine colour and skin elasticity
- pups are best maintained in an incubator (Figure 23.1) in an oxygen-rich environment

Hypoglycaemia

- pups have low glycogen reserves at birth and low functional capacity of the liver
- failure to suck result in hypoglycaemia in 24–36 hours
- hypoglycaemia is serious and sadly response to glucose administration may be poor
- common clinical signs include crying, tremors, irritability, increased appetite, lethargy, coma and seizures
- glucose concentration can be evaluated using a glucose reagent stick or by using a glucometer
- values of <30–40 mg/dl indicate hypoglycaemia and slow intravenous dextrose administration is needed
- dextrose is given at 0.5–1 g/kg as part of a 5–10% solution of dextrose in lactated Ringer's or normal saline, or by giving 1–2 ml of 10–25% glucose intravenously to severely depressed puppies

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NB: Care must be taken to collect only a small sample of blood and use of an insulin syringe is best for this. It is important to keep accurate records when taking blood samples from puppies and to take no more than 10% of the puppy's circulating volume in 1 week.

Hypothermia

- a body temperature less than 35 °C within the first few days is significant
- hypothermia causes reduced gut motility and ileus, leading to a bloated abdomen
- poor respiratory function, poor sucking and increased risk of bacterial infection follow bloating
- reversal of hypothermia requires slow warming, not exceeding 1 °C per hour using heat pads wrapped in towels, heat lamps or bottles, although care must be taken to check the environmental temperature regularly to prevent overheating
- pups should not be fed if their body temperature is less than 35 °C and/or if there are no bowel sounds
- providing dextrose at the same rate as previously described can help meet the increased calorific demand which results from the increase in metabolic rate associated with reheating

Hypoxia

- hypoxia causes a failure to suck, decreases gut motility, and can lead to translocation of intestinal bacteria
- there is also usually a respiratory acidosis because of poor respiratory function
- clinical signs include abdominal distension and increased respiratory effort and rate
- oxygen can be administered using a constant flow oxygen mask or by placing the pup in a 30–40% oxygen environment using an oxygen tent
- a tracheal catheter can be used for endotracheal intubation in pups with upper respiratory tract problems
- intravenous administration of doxapram may stimulate breathing but has not been proven to be efficacious
- artificial ventilation may be useful to encourage lung surfactant production (surfactants are proteins that reduce surface tension of fluid and allows lung alveoli to expand)

Dehydration

- dehydration can occur quickly and be fatal
- fluid should be administered to meet ongoing maintenance requirement (generally 60–100 ml/kg/day or 3–4 ml/kg/hour) as well as to replace the deficit

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- oral electrolytes may be considered in mild cases only when bowel sounds are present; generally intravenous, intraperitoneal or intraosseus administration is required

NB: A typical neonate weighing 0.40 kg will require 1.4 ml/hour maintenance.

Intravenous fluid administration

- the method of choice if possible and it is well worth spending the time to place a catheter even if this requires cut down onto the vessel; this will only become more difficult later on if the pup deteriorates
- over-the-needle catheters are the easiest to keep in position
- the jugular vein may be preferable to the cephalic vein as it is less prone to kinking
- sterile site preparation is necessary

Intraperitoneal administration

- often not used since dosing is required every 8 hours, recurrent needle placement may cause sepsis and fluids are relatively slowly absorbed

Intra-osseus administration

- useful if venous access is not possible; almost certainly preferable to intraperitoneal administration
- usually performed via the medial aspect of the proximal tibia or the trochanteric fossa of the femur (Figure 23.2)
- an intravenous over-the-needle catheter may be used when the cortical bone is soft enough
- the catheter can be fixed and left in position for up to 72 hours; thereafter a new catheter should be placed into a different bone to avoid fluid leakage caused by repeated puncture
- fluid can be administered at the same rate as for intravenous infusion

General principles of fluid administration

- in most cases it should be assumed that there is acidosis and an isotonic crystalloid solution at a dose governed by the hydration status is required
- fluid should be warm but not more than 1 °C above the puppy's current body temperature
- the volume of fluid for immediate replacement should be approximately 3 ml/kg bodyweight per hour and the risks of overhydration can be minimized by regularly weighing
- signs of overhydration are froth at the lips, increased heart rate, laboured breathing, peripheral oedema and seizures

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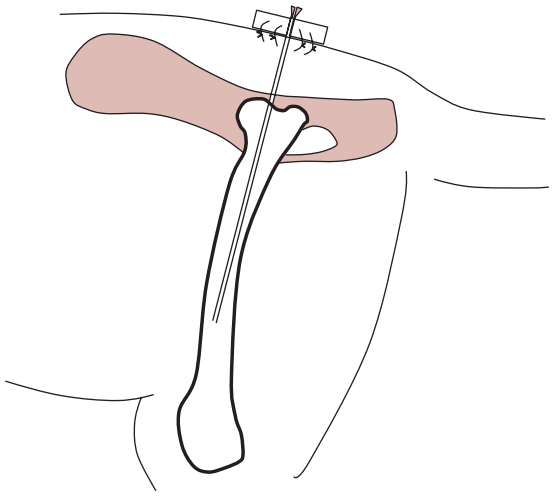


Figure 23.2 Placement of intra-osseus catheter via the trochanteric fossa and held in place with surgical tape and four sutures to the skin.

Commensal bacterial overgrowth

- neonates that are compromised are at increased risk of bacterial infection
- prevention of commensal organism overgrowth can be achieved by administering broad-spectrum antimicrobial therapy, such as clavulanic acid potentiated amoxicillin
- it is always best to take bacteriological swabs but to treat whilst waiting for results

23.5 CAUSES OF DEATH IN NEONATES

- all cases of neonatal mortality should be investigated with postmortem examination since, whilst there are many causes of death, some of these are important infectious organisms which need to be identified so that appropriate control mechanisms can be put in place
- common causes of mortality include:
  (a) congenital defects
  (b) dystocia
  (c) injury
  (d) reduced immunity
  (e) infection (bacterial, parasitic or viral)
  (f) illness or poor nutrition of the dam
  (g) poor maternal behaviour
  (h) poor husbandry
  (i) fading puppy syndrome

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- as discussed appropriate postmortem examination will result in diagnosis of the cause in approximately 50% of cases

Infectious causes of neonatal death

- overall approximately 20% of neonatal deaths result from infection although this can greatly reduced with vaccination programmes and good husbandry (especially vaccination against canine herpes virus where this is endemic) (see 21.5)
- infectious agents include canine distemper virus, canine herpes virus and canine parvovirus. Certain bacteria are able to exploit the vulnerability of the neonate and death can occur rapidly with few clinical signs
- infectious diseases normally progress rapidly and death can be common, but prompt action including testing the bitch for canine herpes virus antibodies and for canine brucellosis and sending samples for microbial culture and sensitivity testing may help prevent illness in the remaining littermates

Fading puppies

- pups that are sick regardless of the cause enter a downward spiral of failure to suck, dehydration and ultimately death; these pups are said to fade
- 'fading puppy syndrome' is, however, a separate condition with similar clinical signs but where the initiating cause is not apparent either clinically or at postmortem examination
- fading puppy syndrome is responsible for 50% neonatal deaths
- affected pups generally appear normal at birth, have an average birthweight, but start to fade from day 2, showing signs of depression, persistent crying, poor sucking response, progressive weight loss and restlessness; most pups die between day 3 and day 5 after birth
- it has been proposed that this syndrome occurs because pups have poor lung surfactant production and therefore do not have adequate respiration which results in a failure to suck
- no treatment has been demonstrated to be effective but in a similar syndrome observed in children improving respiration (using administered surfactant and ventilation) is efficacious; potentially supportive therapy directed towards encouraging respiratory function may be beneficial in pups with the disease

23.6 COMMON CAUSES OF ILLNESS IN NEONATES

- once pups are more than 5 days of age the risk of death from congenital defects and trauma or infections acquired at the time of parturition reduces, however the incidence of diarrhoea and respiratory tract infection increases

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Diarrhoea

- diarrhoea is the most common clinical complaint of neonates and it usually multifactorial in origin
- often there has been limited or absent intake of colostrum, poor hygiene and/or introduction of specific infectious organisms
- often coronavirus, rotavirus and Escherichia coli are isolated and there is commonly secondary bacterial involvement
- the consequences of diarrhoea are rapid dehydration most pronounced in the youngest animals, which can result in a fatal cycle of collapse and death
- whilst the treatment will depend upon the particular cause the mainstay is to consider management of dehydration and hypoglycaemia (see 23.4)

Respiratory disease

- lower respiratory tract disease is not uncommon in puppies and is usually manifest as lethargy, weakness, coughing and anorexia
- it is commonly the anorexia that starts the pup on the fatal cycle of decline and, again, whilst treatment depends upon the particular cause, management of the anorexia at an early stage (tempting pups to eat using warmed foods of high palatability) is crucial to stabilizing these cases

REFERENCE

1 England, G.C.W. (1996) Renal and hepatic ultrasonography in the neonatal dog. *Veterinary Radiology and Ultrasound* **37**, 374–382.

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24 Approach to Infertility Cases

24.1 DEFINITIONS

- there is some confusion around the terminology used to describe whether an animal is able to produce offspring or not, particularly with reference to the term infertility. The following definitions are helpful:
  Fertility: capability of producing offspring
  Sterility: absolute inability to produce offspring
  Infertility: a reduced ability to produce offspring which is below the normal for that species or breed (strictly this word is synonymous with sterility but in recent years its use has changed as indicated)
  Subfertility: a less than average ability to produce offspring; sometimes used synonymously with infertility

24.2 EXPECTATIONS OF FERTILITY

- not all matings between a fertile male and a fertile female result in a pregnancy
- this introduces the concept of pregnancy rate, which is the number of bitches that get pregnant per oestrus cycle they are mated at
- whilst bitches may become pregnant there is also the possibility that the number of pups born can be normal or low
- overall approximately 80% of normal bitches that are mated produce pups and the litter sizes vary greatly in different breeds
- neither bitches nor dogs are often exploited for maximal production, so that ideal total pup output cannot be defined
- in breeding kennels the rate of pup production is usually related to demand, which may be influenced by time of year or other factors
- for the veterinary surgeon the earliest definition of a fertility problem is when the owner of either dog, bitch or kennels is disappointed by results; often the owner has eliminated some of the possible causes so that questions regarding previous breeding history should be put tactfully

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- in general, advice is often sought after a dog has mated three bitches, or a bitch has been mated at two heats, unsuccessfully

24.3 THE INFERTILE BITCH [1]

- in this section common causes by presentation are listed with cross-referencing to the appropriate section within the book. See Figure 24.1

Infrequent oestrus

- delayed puberty (see 19.1)
- long inter-oestrous interval (see prolonged anoestrus 19.5)
- poor observation by owner

Mating difficulties

- inexperienced dog (see 9.4)
- inexperienced bitch (see 9.4)
- psychological problem in the dog (see 9.5 and 20.6)
- psychological problem in the bitch (see 9.5 and 19.10)
- bitch not 'ready' (see 9.2)
- vulval stenosis (see 16.1)
- vestibular constriction (see 16.2)
- vaginal constriction (see 16.3)
- incompatibility of size: this is rarely a serious problem although failure to mate for other reasons may be blamed onto a size difference
- no 'tie' (see 9.4)
- vaginal hyperplasia and protrusion (see 16.3)
- vaginal tumours (see 16.3)

Failure to conceive after normal mating

- mating at wrong time (see 9.2, 11.1 and 19.6) [2]
- mating by infertile dogs
- uterine aplasia (see 16.5)
- tubal lesions (see 16.6)
- vaginal 'infection' is probably not a cause of infertility (see 5.5, 5.10 and 16.3)

Pregnancy failure

- resorption (see 21.3)
- abortion (see 21.5)

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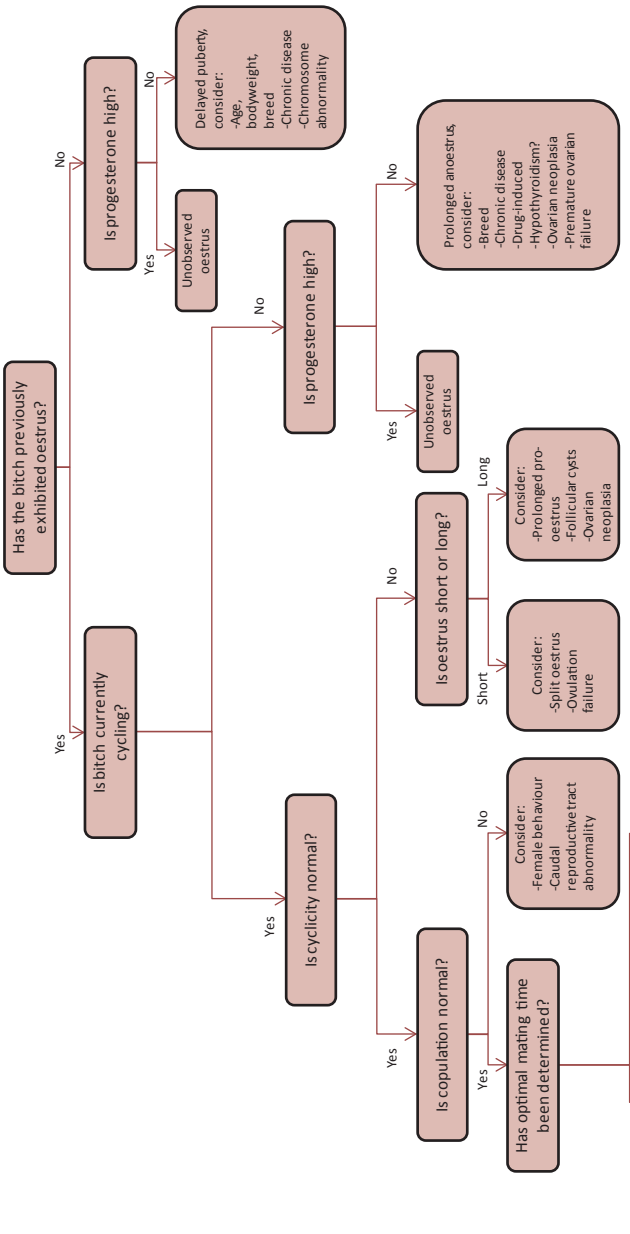


Figure 24.1 Flow chart for investigation of common presentations of infertility in the bitch.

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24.4 TREATMENT OF INFERTILITY IN THE BITCH

Delayed puberty and long inter-oestrus

- consider establishing karyotype
- consider oestrus induction (see below)
- if this is unsuccessful, consider exploratory laparotomy or laparoscopy to check for the presence of ovaries

Oestrus induction

- a variety of pharmacological regimes have been used, however the most effective available in clinical practice are the prolactin inhibitors

Prolactin inhibitors

- apparently normal and fertile oestrous periods may be induced in anoestrous bitches following daily administration of cabergoline
- the time to the onset of induced oestrus seems to relate to the stage of anoestrus; bitches in late anoestrus respond more quickly than those in early anoestrus
- treatment starting in the luteal phase may also be effective, but the treatment period is longer and pregnancy rates are low
- for bitches when treatment starts in anoestrus, the induced oestrus appears to be physiological, and whilst the mechanism of action is uncertain it may be the result of inhibition of remnant progesterone production by the corpora lutea

Gonadotrophin releasing-hormone agonists

- GnRH superagonists (deslorelin) are available and may be useful for initiating release of FSH and LH
- careful attention needs to be paid to the duration of treatment since after initial stimulation there is subsequent receptor down-regulation
- generally deslorelin is administered as an long-acting implant placed subcutaneously; a sero-haemorrhagic vulval discharge usually occurs within 5 days of administration at which point the implant should be removed
- relatively normal pro-oestrus and oestrus follow and pregnancy rates are good although some bitches have a shortened luteal phase that may result in resorption or abortion

Gonadotrophins

- repeated doses of equine chorionic gonadotrophin (eCG) given to anoestrous bitches stimulates follicular growth and the production of oestrogen:
  (a) ovulation may occur spontaneously, following an endogenous surge of LH

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  (b) ovulation may need to be induced using human chorionic gonadotrophin (hCG) which is LH-like in activity
- these regimes are more effective in late anoestrous bitches compared with those in early anoestrus
- care must be observed when using eCG and hCG combinations because endogenous hyperoestrogenism may occur and result in inhibition of implantation, bone marrow suppression and death
- low doses of gonadotrophins are suggested to produce more physiological plasma oestrogen profiles [3]
- a relatively low plasma progesterone concentration or short luteal phase following gonadotrophin-induced oestrus is common; this effect might be mediated by the high endogenous or exogenous oestrogen concentration inhibiting release of the luteotrophic gonadotrophin LH

Oestrogens

- low doses of oestrogen enable follicle-stimulating hormone (FSH) to stimulate the formation of luteinizing hormone (LH) receptors on granulosa cells and therefore increase the responsiveness to basal concentrations of LH:
  (a) the result is follicular growth and production of oestrogen
  (b) gonadotrophin concentrations are low due to the negative feedback effect
  (c) as follicles mature their walls luteinize and progesterone is produced
  (d) the decline in the oestrogen:progesterone ratio facilitates the preovulatory LH surge
- the induction of oestrus with diethylstilboestrol has been used clinically for some time
- in one study when diethylstilboestrol was administered until 2 days after the onset of pro-oestrus and ovulation was ensured by the administration of FSH and LH all bitches became pregnant and whelped normally
- subsequent studies have not, however, been as successful, although more recently diethylstilboestrol alone produced good results

Inability to mate

- ensure that the bitch is properly in oestrus
- eliminate physical obstructions as a cause of pain at the time of mating
- familiarize the bitch with the mating environment
- consider using a different dog that is less (or more) dominant
- consider using an anxiolytic or sedative agent
- consider artificial insemination

Failure to conceive after normal mating

- choose a fertile dog
- consider investigating thyroid function

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- perform a complete pre-breeding investigation including ultrasonography of the uterus to rule out uterine disease such as cystic endometrial hyperplasia
- ascertain that mating occurs at the right time at the next oestrus (see Chapter 11)
- ensure that bacterial colonization of the uterus does not occur by administering a 4–6-day course of a broad-spectrum antibiotic after mating; it has not been proven that this problem occurs (see 16.5) and the aim is to limit proliferation of commensal organisms
- consider exploratory laparotomy to ensure the presence of a normal uterus, oviducts and ovaries
- routine bacteriological screening has little role in investigation of these cases

NB: Since the timing of oestrus and the timing of ovulation during oestrus can be quite erratic in normal bitches, few decisions can be made in the light of the previous breeding history.

24.5 THE INFERTILE DOG

- in this section common causes by presentation are listed with cross-referencing to the appropriate section within the book. See Figure 24.2.

Failure to copulate (see 9.4 and 20.6)

- inexperience
- uncooperative bitch
- psychological reasons
- physical reasons

Failure of conception after normal mating [4]

- failure to ejaculate (see retrograde ejaculation 20.5)
- testicular atrophy due to steroids (see 17.3 and 27.1, 27.2, 27.3) [5]
- spermatogenic arrest (see 17.3)
- testicular aplasia (congenital) (see 17.3)
- testicular degeneration (see 17.3)
- poor semen quality (see 10.5)
- mating infertile bitches (see Chapters 16 and 19)

24.6 TREATMENT OF INFERTILITY IN THE DOG (see 9.3 and 18.6)

Failure to copulate

- eliminate obvious physical reasons
- let the dog run with an experienced bitch

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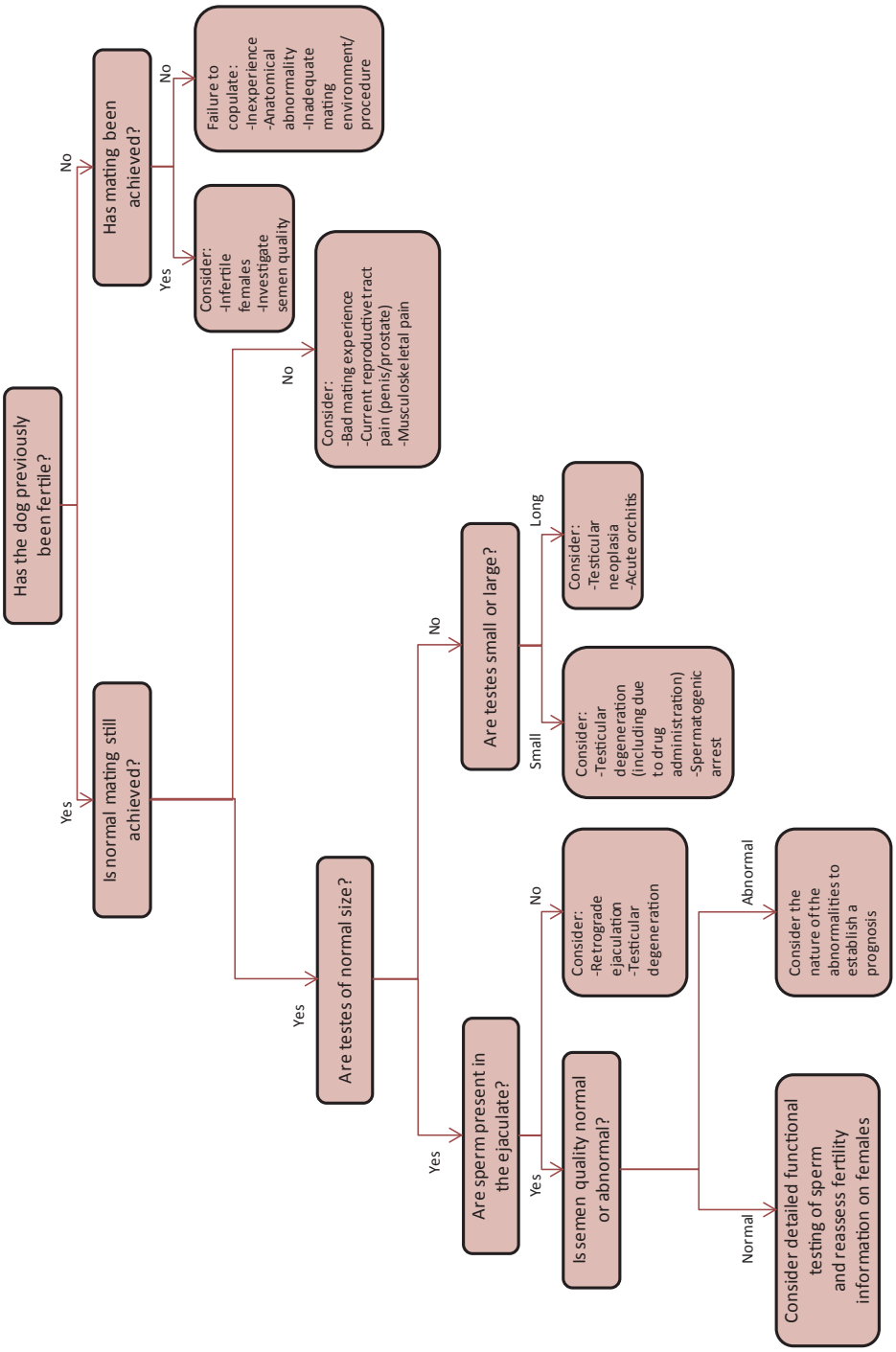


Figure 24.2 Flow chart for investigation of common presentations of infertility in the dog.

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- try a different bitch
- minerals, vitamins and homeopathic remedies have not been shown to be effective but probably do no harm
- do not give androgens or gonadotrophins as they are not effective and may be detrimental to spermatogenesis
- in dogs with musculoskeletal problems having no hereditary component try non-steroidal anti-inflammatory agents

Failure to achieve conception

- ensure that the dog is mated with a 'proven' bitch
- perform breeding soundness examination and collect and evaluate semen quality (see Chapter 10 and 17.3) [4,6]

Morphological abnormalities (teratozoospermia)

- may occur following pyrexia, and resolves quickly (within 60 days) of this event
- minor or major abnormalities in semen quality may be the result of drugs or toxins affecting spermatogenesis or the epididymal phase of development. Drugs known to have a deleterious effect include:
  (a) androgens
  (b) oestrogens
  (c) corticosteroids
  (d) GnRH agonists and antagonists
  (e) ketoconazole
- occasionally seen in the early stages of spermatogenic arrest when the principal abnormality relates to thickening of the mid piece
- an inherited mid piece defect that develops after 4 years of age has been recognized in certain breeds, e.g. springer spaniels
- may occur in the early stages of testicular degeneration secondary to some other pathological process
- in the author's clinic we demonstrated improvement in semen quality by the administration of the synthetic androgen, mesterolone, to these cases [7]

Abnormalities of spermatozoal number (oligozoospermia)

- obstruction of the epididymides or ductus deferens is rare but may be diagnosed by measuring epididymal markers in the ejaculate; there is currently no suitable treatment
- may occur in dogs treated with exogenous steroids that have a negative feedback effect upon the hypothalamus and pituitary gland
- similarly seen in the early stages of dogs with Sertoli cell tumours that secrete oestrogen [8]

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- seen in dogs with testicular hypoplasia, although more commonly these are azoospermic
- seen in dogs with retrograde ejaculation, although usually no fluid is ejaculated in these cases

Absence of spermatozoa (azoospermia)

- seen in the end stage of testicular disease, e.g. following testicular degeneration, autoimmune orchitis, orchitis
- found in dogs with testicular hypoplasia
- found in dogs with bilateral outflow obstruction
- there is no treatment

Abnormalities of spermatozoal motility (asthenozoospermia)

- frequently related to abnormal spermatozoal morphology, i.e. mid piece abnormalities
- may occasionally be seen in dogs with sperm–sperm agglutination
- no suitable treatment has been described

Abnormalities of spermatozoal morphology, number and motility (oligoasthenoteratozoospermia)

- may occur during testicular degeneration
- observed in many of the above listed pathological processes

24.7 INFERTILITY IN KENNELS

The problem

- usually more than one male dog involved, although in many cases the clinical history is more anecdotal rather than accurately documented
- male dogs from outside the kennels (and not known to be infertile) may be involved
- there may be longer than expected interval between successive periods of oestrus
- when matings occur, conception rates are lower than previously expected
- the outcome is that the rate of pup production is significantly reduced
- there may be an increase in the production of dead pups at term
- the death rate of pups during the first week (fading pups) may increase
- the problem may occur gradually or suddenly in established kennels with previous satisfactory fertility

Possible causes

- in some countries the conditions is caused by canine herpes virus infection or Brucella canis infection

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- in the absence of these organisms no other cause been proven; in most cases there may be a combination of aetiologies
- importantly if the condition is associated with abortion, stillbirths and fading pups then testing for canine herpes virus should be instigated

Possible treatments

- eliminate the male dog as a cause, i.e. semen evaluation
- ensure that management factors are adequate; that oestrus is being detected and bitches are mated at the appropriate time
- oestrus induction may be considered where the inter-oestrus interval is prolonged
- investigate general hygiene and puppy care, especially in cases of fading puppies
- when abortion or stillbirths occur pathological investigations of each pup should be performed, serological examination of the bitch may be appropriate; a rising titre after the event may indicate particular infectious organisms
- some veterinary surgeons advocate vaccination for canine herpes virus even if this has not been identified as a cause of the problem

24.8 WHY DO WE KNOW SO LITTLE ABOUT INFERTILITY IN DOGS?

Lack of pressure

- the maximal breeding potential of bitches is rarely exploited due to lack of demand
- compared with other domesticated animals, low fertility is tolerated
- because most dogs are not bred for commercial interests, many infertile animals are not investigated
- dogs are often selected for looks or performance, not for fertility
- usually advice is only sought when the patient is a champion or the 'last of a line'
- owners are often reluctant to admit to a fertility problem in their dogs because they are worried that others will presume the condition is infectious or inherited

Difficulty of investigation in the bitch

- knowledge concerning the significance of vaginal bacteria is usually misinformed (see 5.5 and 16.3)
- digital examination of the whole vagina is impossible, even in large bitches
- visual examination of the whole vagina is very difficult (see 5.7)
- catheterization of the cervix for visualization, swabbing and biopsy of the endometrium is difficult

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- radiography of the vagina and uterus is only possible using contrast media, and information gained from the uterus is not interpretable at the moment
- ultrasonography of the uterus in the absence of gross distension is confined to the distal body where it passes over the bladder (see 5.8)
- ultrasonography of the ovaries is very difficult, but is possible during oestrus; few pathological changes have been described; this situation will shortly change following the author's recent published work on follicular dynamics [9]
- in order to detect hormone abnormalities, bitches would need to be bled at least twice daily over the period when suspected malfunction was to occur; this is usually impractical and expensive
- in order to detect anomalies of pregnancy development, repeated ultrasonographic examinations would be necessary; this would be expensive

Difficulty in investigating the dog

- semen collection and identification of dogs with poor semen quality is easy
- obtaining stud dogs for further investigation, particularly castration and examination of testicular tissue, is very difficult as owners do not usually wish to donate this material
- investigation of possible methods of improving fertility in dogs is difficult because the owners of infertile dogs do not wish them to be used for research

REFERENCES

1 England, G.C.W. and Russo, M. (2006) Conception problems in the bitch. *Journal of Postgraduate Clinical Study In Practice* **28**, 588–597.
2 Hewitt, D.A. and England, G.C.W. (2000) Assessment of optimal mating time in the bitch. *Journal of Postgraduate Clinical Study In Practice* **22**, 24–33.
3 England, G.C.W. and Allen, W.E. (1991) Repeatability of events during spontaneous and gonadotrophin-induced oestrus in bitches. *Journal of Reproduction and Fertility* **93**, 443–448.
4 England, G.C.W. (1995) Evaluation of seminal characteristics in the dog. *Journal of Animal Breeding* **1**, 38–45.
5 England, G.C.W. (1995) Ultrasonographic diagnosis of non-palpalable Sertoli cell tumours in infertile dogs. *Journal of Small Animal Practice* **36**, 476–480.
6 England, G.C.W. and Allen, W.E. (1989) Fertility and semen parameters in dogs. *Veterinary Record* **125**, 399.
7 England, G.C.W. and Allen, W.E. (1991) The effect of the synthetic androgen mesterolone upon seminal characteristics of dogs. *Journal of Small Animal Practice* **32**, 271–274.
8 England, G.C.W. (1991) The relationship between ultrasonographic appearance, testicular size, spermatozoal output and testicular lesions in the dog. *Journal of Small Animal Practice* **32**, 306–311.
9 England, G.C.W., Russo, M. and Freeman, S.L. (2009) Follicular dynamics, ovulation and conception rates in bitches. *Reproduction in Domestic Animals* **44**, 53–58.

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Section 3

Control of Reproduction and Reproductive Technologies

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25 Hormonal Contraception

- unwanted pets are a major social and sometimes ecological problem. They may result in spread of zoonotic disease, cause pollution and damage public areas, livestock and pose an injury risk man. Millions of unwanted pets are destroyed each year
- the permanent solution to controlling unwanted breeding is surgical contraception
- the use of hormones as contraceptive agents is widespread, and these are often given to bitches that are not cycling to prevent the occurrence of the subsequent oestrus, or once pro-oestrus has begun to suppress that particular cycle
- hormonal control of breeding is not widely practised in dogs but details of methods available are given in Chapter 27
- see Chapter 27 for adverse effects of exogenous hormones

25.1 ADVANTAGES OF PREVENTION AND SUPPRESSION OF OESTRUS

- abolishes signs of oestrus and the inconvenience they can cause
- prevents pregnancy
- usually prevents false pregnancy
- is not permanent, i.e. the bitch can be bred from afterwards; although not all regimes are recommended in animals proposed for future breeding

25.2 DISADVANTAGES OF PREVENTION AND SUPPRESSION OF OESTRUS

- requires regular injections or administration of tablets (although this may be overcome with the use of longer-acting implants that are currently in development)
- when used for long periods progestogens can cause side-effects, particularly on the uterus (see 27.1)
- is not a substitute for surgical neutering

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- not recommended for suppression of the first oestrus
- some progestogens, e.g. medroxyprogesterone acetate, may cause mammary neoplasia
- use of GnRH agonists like deslorelin cause initial stimulation which results in signs of oestrus

25.3 SUPPRESSION OF A PRO-OESTRUS THAT HAS ALREADY STARTED [1]

Inject a long-acting progestogen

- progestogens are the only suitable drugs that can be used for oestrus suppression
- for example injection of proligestone early in pro-oestrus is very effective
- signs of pro-oestrus usually disappear within 5 days of administration
- if given late in pro-oestrus, may predispose to pyometra due to increased progestogenic influence on the uterus that has received oestrogen priming and also the cervix will have been 'open' for a longer period of time and as a result bacteria may be present in the uterus
- the next oestrus can be expected 3–9 (average 6) months later

NB: Occasionally an area of alopecia develops over the injection site; for this reason the manufacturers recommend injections on the medial aspect of the flank-fold in show dogs.

Administer an orally active progestogen

- for example, an 8-day course of megestrol acetate commencing early in pro-oestrus
- signs of pro-oestrus usually disappear within 2–3 days after the start of the course
- if given late in pro-oestrus it is theoretically less likely than a long-acting progestogen to predispose to pyometra because the course of drug administration is shorter
- oestrus may recur any time after 4 months later

25.4 PREVENTION OF AN EXPECTED PRO-OESTRUS/OESTRUS [1]

- sometimes an expected oestrus is likely to coincide with a social or another event which makes management of the oestrous bitch difficult, or the owner is planning long-term contraception

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- treatment should be given in anoestrus, i.e. more than 2 months after the end of the previous oestrus; because the length of anoestrus is very long and variable in the bitch, the timing of the treatment is governed by the time when oestrus is to be avoided

Subcutaneous administration of depot preparations during anoestrus

- depot progestogens (medroxyprogesterone acetate, delmadinone acetate, proligestone) administered during anoestrus will prevent a subsequent oestrus
- medroxyprogesterone acetate and proligestone are licensed for this purpose in the bitch

Oral administration of progestogens during anoestrus

- low doses of orally active progestogens (megestrol acetate, medroxyprogesterone acetate, norethisterone acetate) can be used to prevent oestrus for as long as administration is continued
- megestrol acetate and medroxyprogesterone acetate are licensed for this purpose in the bitch
- should the animal enter pro-oestrus during the first few days of treatment, the dosage can be increased
- a period of anoestrus usually follows therapy so oestrus does not return immediately after cessation of treatment

Oral administration of synthetic androgens during anoestrus

- a synthetic androgen, mibolerone, which is not available in the UK, is effective for the prevention of oestrus in bitches
- mibolerone is formulated as a liquid for daily oral administration
- mibolerone has adverse effects typical of other androgens, including clitoral hypertrophy, vaginitis and behavioural changes; in addition, anal gland abnormalities, obnoxious body odour and obesity have also been recorded

Administration of depot androgens during anoestrus

- administration of testosterone either as a prolonged release implant, or depot injection of mixed testosterone esters may be used to prevent oestrus in bitches

Administration of a GnRH agonist

- administration of a high-dose GnRH superagonist implant (deslorelin at approximately 5 mg) may be suitable for preventing recurrence of oestrus in bitches when they are implanted in deep anoestrus

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- there may be period of behavioural oestrus shortly after implantation because of the initial stimulation produced before receptor down-regulation
- implants need to be inserted approximately every 5 months
- adverse effects are minimal

NB: Deslorelin implants used for oestrus induction are removed when signs of pro-oestrus occur, whereas for oestrus suppression the implants are left in place.

25.5 PREVENTION OF REPRODUCTIVE BEHAVIOUR FOR EXTENDED PERIODS OF TIME [1]

It may be convenient to prevent reproductive behaviour for a long period of time when the ultimate aim is still to breed from the bitch.

Subcutaneous administration of depot preparations during anoestrus

- medroxyprogesterone acetate and proligestone are licensed for this purpose in the bitch
- they are normally given at 3, 4 and thereafter 5 month intervals for proligestone, and 6 month intervals for medroxyprogesterone acetate
- it may be inadvisable to prevent oestrus for more than 2 years, but providing that a breakthrough cycle does not occur, prolonged prevention may have few adverse effects, especially when using the more recently developed progestogens (proligestone); it has been reported that incidence of pyometra and mammary tumours is reduced compared with untreated females
- when therapy is ceased most females cycle normally, although progestogens may induce cystic endometrial hyperplasia and therefore reduce fertility

Short courses of orally active progestogen

- for example, megestrol acetate courses are given at the beginning of each pro-oestrus
- the advantage of such treatment is that progestogen is only given for short periods of time; in some bitches this may be as infrequently as every 8–12 months
- the disadvantages are that signs of pro-oestrus must be looked for, and the inconvenience of a few days of pro-oestrus must be endured
- other possible side-effects are discussed in 27.1
- a period of anoestrus usually follows therapy so oestrus does not return immediately after cessation of treatment

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Oral or depot administration of androgens during anoestrus

- mibolerone is effective for the long-term prevention of oestrus in bitches
- often depot androgen therapy (e.g. mixed testosterone esters) is supplemented by daily oral therapy, e.g. testosterone esters are given intramuscularly every 4–6 weeks, with supplemental daily oral testosterone
- adverse effects are more common with prolonged therapy
- androgens are given at regular intervals to greyhound bitches to prevent oestrus because:
  (a) bitches in oestrus cannot race because they would attract male dogs
  (b) bitches in early metoestrus, i.e. for 10 weeks after the beginning of pro-oestrus, should not race because the high circulating concentrations of progesterone at this time depress performance and make racing predictability difficult
- the possible anabolic effects of these preparations appear not to be marked enough to enhance racing performance
- there are no known untoward effects on subsequent fertility (see 27.3)
- drugs must not be administered to greyhounds during the 7 days before racing

Administration of a GnRH agonist

- repeated administration of deslorelin implants approximately every 5 months appears to be reasonably effective as described above but these products have not received marketing authorisation for this purpose

25.6 FUTURE METHODS OF PREVENTING OESTRUS

Immunization

- immunization of bitches against zona pellucida proteins, gonadotrophins and GnRH may provide an effective means of contraception
- a reliable GnRH protein conjugate antigen has recently been developed
- such methods require further investigation before they become clinically useful

25.7 UNWANTED MATING

Definitions

Misalliance: is defined in man as an unsuitable marriage
Mesalliance: is defined as an unsuitable marriage between two people of different social backgrounds

NB: In dogs either term is used to describe an unwanted or unplanned mating; probably these are more appropriate terms.

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The clinical problem

- typically the bitch is presented with a history of:
  (a) having been caught 'tied' with a dog
  (b) having been 'seen' with a male dog
  (c) having escaped for some time and perhaps returned with saliva on her neck, etc.
- therefore in many cases it is not known whether mating has occurred or not; a study in the USA suggested that 50% of bitches presented for unwanted mating had not actually been mated
- treatments for unwanted mating either have potential adverse effects or are costly

Examination of the bitch following presumed unwanted mating

- the 'blind' treatment of all bitches presumed to have had an unwanted mating may, in certain circumstances, be appropriate
- a more satisfactory approach is the careful examination of bitches and the application of treatment in appropriate cases
- clinical evaluation of the bitch should involve:
  (a) the collection of a vaginal smear to evaluate the stage of the oestrous cycle and to try to detect spermatozoa
  (b) the use of a modified vaginal smear technique to detect spermatozoa
  (c) the measurement of plasma concentration of progesterone to establish accurately the stage of the oestrous cycle

Vaginal cytology

- collection and study of exfoliative vaginal cells may determine the stage of the oestrous cycle (see 5.5)
- these results should be used in combination with measurement of plasma concentration of progesterone
- in recently mated bitches, spermatozoa may be identified within the vaginal smear
- there is however a rapid reduction in the number of spermatozoa and they may not be identified if the interval from mating exceeds 24 hours
- three possible cytological classifications occur:

Bitches that are found to be in cytological pro-oestrus

- in these cases plasma progesterone concentrations are low
- such bitches may not have been mated, since mating is usually refused at this time, and therefore do not warrant treatment
- however, this is not always the case, and some early matings may result in a pregnancy if the spermatozoa are able to remain viable within the female reproductive tract until after ovulation

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- assessment of the presence or absence of spermatozoa is therefore essential in these cases

Bitches that are found to be in cytological oestrus

- in these cases plasma progesterone concentration is usually intermediate or high
- such bitches may have been mated, and in most cases should receive treatment
- reference to the presence or absence of spermatozoa may influence whether treatment is instituted

Bitches that are found to be in cytological metoestrus (dioestrus)

- in these cases plasma progesterone concentration is high
- such bitches may have been mated and should be examined for the presence of spermatozoa
- the most important factor is, however, the time interval from mating to examination, because it is rare for matings occurring after the onset of cytological metoestrus (dioestrus) to be fertile

Plasma progesterone measurement

- plasma concentrations of progesterone can be measured by ELISA techniques (see 5.9)
  (a) concentrations are low during anoestrus and pro-oestrus
  (b) concentrations increase when pre-ovulatory follicular luteinization occurs
  (c) there is a rapid rise in progesterone concentration after ovulation
- progesterone concentrations should be interpreted in the light of the clinical history and assessment of vaginal cytology

Detection of spermatozoa within the vagina

- from 24 hours after mating there are few spermatozoa within the vagina
- accurate detection of spermatozoa requires a modified cytology technique
- a saline-moistened swab should be placed into the vagina for 1 minute
- after removal the swab tip should be placed in a small test tube with 0.5 ml physiological saline, and allowed to stand for 10 minutes
- the swab is then squeezed dry, and the test tube containing the saline is centrifuged at 2000 rev/min for 10 minutes
- collection of the sediment and microscopic examination after staining with a modified Wright–Giemsa stain allows detection of sperm heads
- using this technique, spermatozoa are found in 100% of samples in which mating occurred within the previous 24 hours, and in 75% of samples in which mating occurred within the previous 48 hours

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Table 25.1 The risk of conception (low, medium, high) in relation to vaginal cytology and plasma progesterone findings for bitches that have had a presumed unwanted mating.

	Pro-oestrous vaginal smear + no sperm	Pro-oestrous vaginal smear + sperm	Oestrous vaginal smear + no sperm	Oestrous vaginal smear + sperm	Met-oestrous vaginal smear + no sperm	Met-oestrous vaginal smear + sperm
Low plasma progesterone	Low	Medium	Medium	Medium	n/a	n/a
Intermediate plasma progesterone	Low	High	Medium	High	Medium	High
High plasma progesterone	n/a	n/a	Low	High	Low	Medium

Treatment options

- there are several treatment options, even when mating has occurred at a time likely to result in conception:
  (a) if the bitch is not required for breeding ovariohysterectomy should be performed in week 4 after the end of oestrus (at a time when pseudopregnancy will not be induced)
  (b) the bitch may be treated in an attempt to prevent implantation
  (c) the bitch can be re-examined approximately 28 days after mating to establish if a pregnancy is present before instituting therapy. If a pregnancy is identified it may be terminated by inducing resorption or abortion
- treatment options can be considered with respect to the risk of conception (Table 25.1)

Prevention of implantation

Progesterone antagonists

- aglepristone is a progesterone receptor antagonist that has a greater affinity for the receptor than progesterone does; administration displaces progesterone from its receptor so there is no biological action of normal progesterone concentrations
- in essence the effect of progesterone which would normally maintain the developing conceptus is removed and the pregnancy does not establish
- aglepristone is administered in two doses 24 hours apart; in giant breeds a high volume of drug needs to be injected and this has to be distributed into several injection sites

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- apart from injection site reactions there are few adverse effects; in most cases the bitch appears never to have been pregnant

Oestrogens

- oestrogens are thought to have several effects that prevent implantation:
  (a) they may alter the transport time of zygotes
  (b) they may impair implantation
  (c) they may cause luteolysis by an uncertain mechanism
  (d) they may stimulate uterine contractions and cervical relaxation
- diethylstilboestrol injection was used extensively and apparently successfully in the UK until recently; it is no longer available
- oral ethinyl oestradiol may also be effective
- low dose oestradiol injected on day 3 and 5 (and also possibly 7) after mating, has been found to be clinically effective and has a failure rate and complication rate that is deemed acceptable
- approximately 9% of bitches treated with oestrogen develop pyometra within the subsequent 4 months
- much of the information on oestrogen treatment in bitches appears to be anecdotal
- little scientific interest has been shown into the mode of action of the oestrogens, the optimal dose rate and time of treatment to prevent conception, and the rate of success
- clinically, oestrogen seems to be effective at preventing conception, but:
  (a) many bitches that are treated were probably not mated
  (b) some bitches may have been mated at a time when conception could not occur
- possible reasons for treatment failure:
  (a) earlier mating than was originally thought, so that treatment is too late
  (b) the shorter half-life of oestradiol compared with diethylstilboestrol, may in some cases allow its effects to have worn off by the time that ova enter the uterine tubes; dog sperm can probably remain fertile for up to 7 days in the bitch genital tract (see 11.2)
  (c) the bitch may be mated again after treatment: in most cases, if the oestradiol is given around the ovulation period, this will not matter because even though oestrus may be prolonged, no new ovulations will be stimulated; if, however, a bitch is treated in pro-oestrus, the drug may have been metabolized by the time of second mating and ovulation
- second matings should, however, be discouraged in treated dogs as these may introduce bacteria into the uterus and predispose to pyometra
- repeat injections during the same heat should be discouraged as these probably increase the chances of side-effects (see 27.2)
- adverse effects of oestrogens include:
  (a) oestrogens may potentiate the stimulatory effects of progesterone on the uterus producing cystic endometrial hyperplasia

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(b) oestrogens cause cervical relaxation, thus allowing vaginal bacteria to enter the uterus
(c) these two events may result in the development of pyometra
(d) oestrogens also produce dose-related bone marrow suppression
(e) other effects include alopecia, skin hyperpigmentation and mammary and vulval enlargement

NB: It is prudent to perform a pregnancy diagnosis at 28 days post mating after any treatment to prevent implantation to ensure that pregnancy is not maintained; if it is then the treatment options below are available.

Termination of pregnancy

- once pregnancy has become established it may be terminated either by altering the endocrine environment or by ovariohysterectomy which may be safely performed in week 4 after the end of oestrus; surgery at this time is unlikely to induce a pseudopregnancy
- for medical termination of pregnancy it is important to recognize that the bitch is dependent upon the ovarian production of progesterone throughout gestation
- methods which produce luteolysis may therefore be used to terminate pregnancy
- prolactin is the most important luteotrophic agent in the dog; therapy may also be directed against this hormones to cause demise of the corpora lutea and the termination of pregnancy

Progesterone antagonists

- aglepristone displaces progesterone from its receptor but does not change progesterone concentrations in the blood; the progesterone has no biological action and therefore the pregnancy will be terminated
- aglepristone is administered in two doses 24 hours apart; in giant breeds a high volume of drug needs to be injected and this has to be distributed into several injection sites
- the regime can be implemented at any time:
  (a) before day 21 there are no obvious clinical outcomes other than the bitch does not stay pregnant
  (b) between day 21 and day 35 there is commonly a haemorrhagic discharge but with few other signs
  (c) between day 35 and day 45 there is normally a haemorrhagic discharge and some fetal components may be expelled, the bitch has uterine contractions and signs similar to parturition
  (d) after day 45 there is a variable response and there are some reports of fetal death but no expulsion; administration of prostaglandin may be necessary for elimination of fetal material

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- later treatments are generally perceived to be less acceptable by the owner and the possibility of failure of expulsion (because this drug regime does not cause active uterine contractions) makes early treatment most appropriate

Combination of prolactin inhibitor and prostaglandin

- whilst it is true that both prolactin inhibitors and prostaglandins may be used alone to terminate pregnancy, treatment efficacy is improved and (at least for prostaglandins) dose can be reduced which reduces adverse effects
- the combination of the dopamine agonist cabergoline given daily for 10 days and the synthetic prostaglandin cloprostenol given every other day, will induce pregnancy termination from day 28 after ovulation onwards
- earlier treatment is not efficacious as cabergoline inhibits prolactin release (and there is insufficient prolactin until that time) and prostaglandins are more effective in causing luteolysis after day 25
- low doses of cabergoline are used (5 µg/kg/day)
- low doses of cloprostenol are used (2–5 µg/kg); these are much lower than early publications using prostaglandin describe, and as a result adverse effects such as salivation, vomiting, pyrexia, hyperpnoea, ataxia and diarrhoea are either mild or do not occur
- prostaglandin is also useful because it causes uterine contraction and helps to dislodge the pregnancy
- when bitches are treated for approximately 10 days, 100% will resorb, and there are generally no adverse effects

Corticosteroids

- in many species glucocorticoid administration during pregnancy induces abortion
- in the bitch repeated administration and high doses of dexamethasone are required
- the method has found limited use in clinical practice

NB: After any attempt to terminate pregnancy the bitch should be examined with ultrasound to confirm complete resorption or expulsion.

25.8 HORMONAL CONTRACEPTION IN DOGS

- there is less use of hormonal contraception in males, especially since methods used to suppress testosterone production (and therefore to control male behaviour) also have an effect upon spermatogenesis; for animals required for breeding in the future these methods are therefore often avoided
- male behaviour and contraception can be controlled by the administration of progestogens, androgens and combinations of these; in the author's laboratory we have shown these to be highly efficacious [2] (Figure 25.1)

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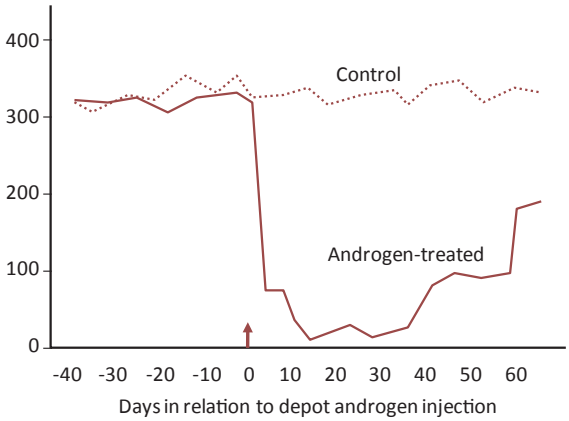


Figure 25.1 Mean total sperm output from dogs treated with a single depot administration of androgen and from control dogs. There is a rapid reduction in semen quality to almost azoospermia in most treated dogs within 2 weeks.

- contraception can also be achieved by administration of long-acting implants containing a GnRH agonist; this causes down-regulation of receptors and a reduction in secretion of LH and FSH and therefore decreased testicular steroidogenesis and spermatogenesis
- further details are given in Chapter 27

REFERENCES

1 England, G.C.W. (1994) Hormonal manipulation of breeding in the bitch. *The Veterinary Annual* **34**, 189–200.
2 England, G.C.W. (1997) Effect of progestogens and androgens upon spermatogenesis and steroidogenesis in dogs. *Journal of Reproduction and Fertility* **51**(Supplement), 123–138.

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26 Surgical Neutering

26.1 OVARIOHYSTERECTOMY OF THE BITCH

- ovariohysterectomy (neutering) means removal of the uterus and both ovaries; this is the standard practice in many countries although it is gradually being replaced by ovariectomy (removal of the ovaries only)
- in bitches not intended for breeding this operation should be considered seriously because, in most cases, any disadvantages are far outweighed by the advantages; it also helps to reduce the problem of unwanted pups
- there is no evidence that surgical neutering has a beneficial effect on behaviour
- there is a risk of dominance aggression in bitches spayed at a young age when there are already signs of aggression before the surgery
- removal of the ovaries causes a slight increase in appetite in some bitches

Indications

- removal of the ovaries prevents problems associated with oestrus including:
  (a) vulval discharge, repeated urination and unwanted attention of male dogs whilst exercising the bitch
  (b) nuisance and road traffic accidents caused by the bitch roaming and male dogs being attracted to the house
- prevents unplanned pregnancies
- prevents pyometra (see 16.5)
- should prevent false pregnancy (see 3.7 and 19.8)
- reduces the incidence of mammary neoplasia (bitches which are neutered before the first oestrus very rarely develop mammary neoplasms)

Timing

- spaying bitches before puberty is surgically easiest and should reduce the likelihood of operative complications

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- surgery at this time will reduce the incidence of subsequent mammary neoplasia and there is no risk of pregnancy
- however, it is proposed that pre-pubertal surgery may increase the incidence of obesity, incontinence and 'juvenile' vulva (see 16.1), although this has not been proven
- most authorities now recommend pre-pubertal neutering but there are some clinical situations when this may not be suggested:
  (a) bitches that have a current pre-pubertal vaginitis; as this may not resolve until after the first oestrus
  (b) bitches that have current signs of urinary incontinence; as this may worsen with pre-pubertal neutering
  (c) bitches with underdeveloped external genitalia; as these may not develop normally after neutering
  (d) bitches that have signs of dominance aggression; as this may worsen following pre-pubertal neutering
  (e) bitches from breeds where coat colour changes may occur after pre-pubertal neutering (several breeds with red-coloured hair); as this may be exacerbated by pre-pubertal neutering

Timing of surgery after the first oestrus

- there are two clear windows at which time neutering is appropriate after oestrus

Approximately 20 days after oestrus

- this is a good time as the risk of inducing a pseudopregnancy is small (as prolactin concentrations are not yet elevated)
- the blood supply to the uterus and ovaries is good at this time and haemostasis may be a problem
- this may be a good time to spay a bitch which is not wanted for breeding and has been mated inadvertently

From 3 months after oestrus to just before the next pro-oestrus

- by this time the ovaries (corpora lutea) have become inactive and blood supply is minimal

Other points relating to the time of surgery

- after parturition; a bitch can be spayed as soon as the puppies are completely weaned (6–8 weeks) and lactation has finished
- it is not sensible to perform the surgery between 5 weeks and 2.5 months after oestrus since at this time progesterone concentrations are maintained by prolactin so that removal of the ovaries results in a reduction of progesterone and

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a dramatic increase in prolactin and therefore the induction of iatrogenic pseudopregnancy
- do not operate if a bitch is in false pregnancy as signs may continue post-surgically
- cases of pyometra must be operated on when the patient's chances of survival are greatest despite the fact that surgery at this time may induce a pseudopregnancy

Technique

- the detailed technique is available in other standard textbooks

Complications of ovariohysterectomy

- surgical and immediately post-surgery complications are described in other standard textbooks

Haemorrhagic discharge per vaginam

- a bloody vulval discharge within 7 days of surgery is usually due to a transfixing ligature being placed through the vaginal lumen;
- rarely it may be associated development of a uterine granuloma; infection at this site can be a serious complication
- mild cases can be treated symptomatically, although if haemorrhage is persistent and severe it will be necessary to re-operate and remove the vaginal stump

Post-renal granuloma with a discharging sinus in the flank

- due to using non-absorbable ligature material which was contaminated with bacteria
- ensure that the sinus originates from the site of the ovarian pedicle by surgical dissection or contrast radiography: these sinuses may take up to a year to form and so sometimes are not recognized as a complication of the surgery
- remove the abscess by blunt and blind dissection via a mid line laparotomy; this can be very difficult

Signs of oestrus after spay (due to leaving an ovary in situ)

- many cases present because the female is attracting males
- a red-coloured discharge will only be present if the uterus or a portion of it has been left behind (as red cells leak through the endometrium by diapedesis)
- it is always best ensure that the bitch is really in oestrus by taking a vaginal smear and looking for anuclear epithelial cells; some bitches with vaginitis are thought to be in oestrus (see 5.5)

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- if there is confusion about whether a remnant ovary is present a blood sample for progesterone can be taken 4 weeks after oestrus; high progesterone will only have originated from an ovary
- surgery performed at this time will give the greatest chance of locating the ovary which is at its largest size at approximately 4 weeks post oestrus
- if the bitch is presented months after the signs of oestrus have been observed then ovarian remnant can be confirmed by detecting a rise in oestrogen after a GnRH stimulation test
- the ovary should be located within the fat pad caudal to the kidney

Post-spay false pregnancy

- most commonly this occurs because of inappropriate timing of ovariohysterectomy; removal of the ovaries when corpora lutea are present results in a decline in progesterone concentrations and an increase in prolactin concentrations
- these cases generally develop clinical signs within 1–2 weeks of the surgery
- the removal of the ovaries (containing corpora lutea) results in a fall of progesterone and a rise in prolactin
- if pseudopregnancy occurs immediately after surgery it is almost certain that an ovarian remnant is not present
- pseudopregnancy may occur with an ovarian remnant but normally only follows signs of oestrus
- treatment of iatrogenic pseudopregnancy requires prolactin inhibitors and sometimes an extended treatment period of 3 weeks is required to ensure a cure

Obesity

- this is the most common post-spay problem
- some breeds are likely to put on weight anyway after middle age, e.g. Labrador retrievers
- it is easier to prevent a dog from gaining weight than it is to reduce its weight
- in order to prevent weight gain:
  (a) advise the owner of this likelihood as often as possible
  (b) advise regular weighing of the bitch after spaying
  (c) consider the early use of 'obesity diets' if reduction of energy intake and increased exercise do not prevent weight gain

Change of coat texture

- for example, Irish setters, Samoyeds, etc. These changes are unavoidable

Urinary incontinence (see 19.10)

- most commonly there is an overflow incontinence seen when the bitch is recumbent

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- signs may develop even within a few weeks of the surgery, although in other cases signs may take months or years to appear
- the incontinence appears to be associated with:
  (a) atrophy of the urethral muscles and epithelium which results in an insufficient sphincter mechanism and allows the uncontrolled escape of urine
  (b) rarely an acquired ectopic ureter occurs due to the ureter being inadvertently included in the uterine ligature and forming a fistula into the vagina; the possibility of this occurring seems very unlikely and cases have been recorded where laparotomy with no involvement of the urogenital tract has apparently triggered off' incontinence due to an ectopic ureter

Pyometra

- occasionally, if the uterine body is not removed a pyometra can develop, i.e. a 'stump pyometra'
- a true pyometra condition can only occur if the remnant uterus is exposed to ovarian hormones (either oestrogen or progesterone or both); this means with an ovarian remnant needs also to be present or the bitch needs to be given exogenous hormones before the condition can develop (many clinical cases are actually uterine granulomas and not a true pyometra)
- exogenous oestrogen may be given to spayed bitch to control signs of urinary incontinence
- exogenous progestogens are rarely given to spayed bitches
- initially the condition may not be recognized because the owner and veterinary surgeon do not expect the bitch to have a uterus as she has been 'spayed'
- the treatment is as for pyometra

26.2 OVARIECTOMY OF THE BITCH

- in many European countries ovariectomy rather than ovariohysterectomy has been performed for many years
- there is a growing trend for pre-pubertal ovariectomy to be performed by laparoscopy
- after removal of the ovaries the uterus rapidly atrophies
- uterine disease is uncommon unless the bitch is given exogenous reproductive steroids, e.g. progestogens for the control of skin disease, or oestrogens for the control of vaginitis
- the laparotomy surgical technique is more simple and can be performed quickly compared with that required for an ovariohysterectomy
- the laparoscopic technique requires special training but when perfected can be performed quickly and safely; during the surgery the ovary is fixed to the lateral abdominal wall to help vessel ligation. Recovery from surgery is remarkably pain free a few hours after the surgery – this will become the method of choice for surgical neutering within the next few years

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26.3 ORCHIDECTOMY (CASTRATION) OF THE DOG

- orchidectomy (orchiectomy, castration, neutering, gelding) means removal of the testes and the epididymides
- neutered male dogs make better pets because they are less likely to roam or be restless; they are not distracted by bitches in oestrus and they are unable to produce puppies

Indications

- castration reduces the incidence of many sex-linked antisocial habits, e.g. copulatory behaviour, roaming, urine marking and aggression to other male dogs

NB: Castration rarely modifies aggression towards people which is often motivated by fear.

- castration is required in cases of testicular or scrotal trauma or pathology
  (a) testicular tumours (see 17.3)
  (b) testicular torsion (see 17.3)
- castration is necessary in dogs that are cryptorchid or have ectopic testes (see 17.3)
- castration may be necessary to control disease stimulated by testosterone
  (a) prostatic disease (see 17.7)
  (b) peri-anal adenoma
  (c) perineal hernia

Timing

Elective castration

- this may be carried out from 5 months of age; early castration may increase the likelihood of obesity and delay closure of growth plates resulting in animals having a long-legged stature

Castration for antisocial behaviour

- this may be carried out at any time; it is most effective for the prevention of roaming
- older dogs are more likely to have 'learned' behavioural patterns, i.e. they are no longer testosterone dependent and these show limited response to surgery
- castration may be preceded by progestogen or GnRH agonist treatment to assess the likely efficacy of surgery; where medical treatment shows a good response it is likely that surgery and behavioural modification therapy will be able to permanently control the exhibited behaviours

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Cryptorchid and ectopic testes

- testes that are held at a higher temperature than is present within the scrotum are more likely to become neoplastic than scrotal testes
- they should be removed before the dog is about 3 years old

NB: Unilateral cryptorchid dogs are fertile and potent; bilateral cryptorchids are sterile and potent (see 17.3).

Technique

- the detailed technique is available in other standard textbooks

Complications of castration

- surgical and immediately post-surgery complications are described in other standard textbooks

Scrotal swelling

- this is a common sequel because of the 'dead' space left within the scrotal pouch
- swelling is usually caused by blood and/or inflammatory fluid and may give the impression that the testes have not been removed
- some cases are severe and described as scrotal haematoma
- scrotal swelling can be minimized by strict haemostasis, whilst some veterinary surgeons ablate the scrotum as part of the procedure in adult dogs of large breeds
- these cases are best managed with antibiotics to prevent infection, use of an Elizabethan collar to prevent trauma to incision and scrotum, and assurance of the owner that the swelling is not due to growth of new testicles!

Aggression

- some owners worry that the dog may become more aggressive: this is not true
- the temperament change that occurs in most pets is desirable and not detrimental
- in working dogs, however, castration may significantly affect performance

Obesity

- this does not seem to be such a significant problem as seen in the bitch
- dogs that are castrated before puberty may develop a female-like shape

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26.4 VASECTOMY

- removal of a section of the vas deferens is commonly performed to create sterility but without an impact upon reproductive behaviour in several animal species including man
- there are no reasons for vasectomy to be undertaken in dogs since it has none of the additional benefits of castration in terms of protection of reproductive tract disease and controlling male-stimulated behaviour
- vasectomy is not normally performed in dogs

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27 Pharmacological Control of Reproduction

27.1 PROGESTOGENS [1]

- progesterone is produced by the corpora lutea of the bitch, and is not naturally produced by the dog
- endogenous progesterone suppresses spontaneous myometrial activity, causes closure of the cervix and stimulates endometrial growth and secretion
- progesterone is responsible for mammary gland development during the luteal phase
- progesterone has a feedback effect upon the hypothalamus and pituitary gland
- progestogens are compounds with progesterone-like activity, and therefore they each exhibit the actions above:
  - (a) it is, however, their negative feedback effect upon the hypothalamus and pituitary gland that is the principal reason why they are so widely used for the control of reproduction
  - (b) progestogens appear to exert many of their actions by preventing a rise in gonadotrophin secretion
  - (c) progestogens have a negative feedback effect upon the release of prolactin, and can reduce, to some extent, circulating concentrations of testosterone and oestrogen by their actions upon gonadotrophin secretion
- high doses of progesterone and progestogens are centrally sedative in action
- in male dogs progestogens have an additional and direct effect upon the prostate gland causing a reduction in size, mediated probably by blocking the action of androgens (they are therefore described as being anti-androgenic in addition to their effects in reducing testosterone concentrations)
- progesterone and progestogens are commercially available in a variety of formulations including oral therapy that must be given daily, and oily suspensions and implants that provide a slow release over several weeks or months

Adverse effects of progestogens

General

- many transient effects may follow the administration of progestogens including:

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(a) increased appetite
(b) weight gain
(c) lethargy
(d) mammary enlargement (and nodules and possible neoplasia with first-generation progestogens)
(e) occasional lactation (following the withdrawal of the agent)
(f) hair and coat changes
(g) temperament changes

- these effects vary in their incidence between the different progestogens, although in general they are less frequent with the more recently developed ones
- the subcutaneous administration of some progestogens (especially the depot preparations) may produce hair discoloration and local alopecia at the site of injection
- it is therefore recommended that administration should be performed in an inconspicuous site, although this is usually impractical
- all progestogens may potentially induce the production of growth hormone. Chronic over-secretion of growth hormone may result in the clinical signs of acromegaly and peripheral insulin antagonism which may result in diabetes mellitus
- progestogen therapy may also produce adrenocortical suppression

Specific adverse effects in the bitch

- progestogens may result in the development of cystic endometrial hyperplasia and pyometra (see 16.5)
- the risk is related to the particular progestogen used, as well as the amount administered and the duration of treatment
- this action appears to be potentiated by oestrogen, and for this reason certain depot progestogens (medroxyprogesterone acetate) are not licensed for use when oestrogen concentrations are elevated (they are therefore not used for the suppression of oestrus)
- other depot progestogens (proligestone and delmadinone acetate) have been shown to be safe when administered at practically any stage of the oestrous cycle
- there are no preparations recommended for use at the first oestrous period or in pre-pubertal bitches
- benign mammary nodules can be induced by progestogen therapy, and it has been suggested that progestogens may induce mammary neoplasia, although this does not appear to be the case with proligestone
- progestogen therapy during pregnancy may delay or prevent parturition, and may produce masculinized female and cryptorchid male fetuses

Specific adverse effects in the dog

- high doses of progestogens cause marked changes in semen quality, the majority of which are related to a direct effect upon the epididymides
- changes in semen quality may result in infertility

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- however, it is surprising that lower doses administered for short periods of time produce no measurable effect on semen quality or fertility

Clinical use of progestogens in bitches

Control of oestrus (see Chapter 25)

- administration of progestogens to bitches in anoestrus prevents gonadotrophin secretion increasing above basal values, and therefore prevents a return to cyclical activity
- if given when there is follicular activity (pro-oestrus or early oestrus), ovulation is inhibited and the bitch returns relatively quickly to anoestrus
- when progestogens are given daily or as a depot preparation they mimic a luteal phase, which is followed by a state of anoestrus
- progestogens are generally administered in one of four regimes to control oestrus in the bitch:

Subcutaneous administration of depot preparations during anoestrus

- depot progestogens (medroxyprogesterone acetate, delmadinone acetate, proligestone) administered subcutaneously during anoestrus will prevent a subsequent oestrus and regular repeated dosing (at 4–6-month intervals) can be used to prevent oestrus on a long-term basis
- medroxyprogesterone acetate and proligestone are licensed for this purpose
- delmadinone acetate is unlicensed and has to be administered more frequently because of its shorter duration of action
- it may be inadvisable to prevent oestrus for more than 2 years
- when therapy is ceased most females cycle normally
- progestogens may induce cystic endometrial hyperplasia and therefore reduce fertility

Oral administration of progestogens during anoestrus

- low doses of orally active progestogens (megestrol acetate, medroxyprogesterone acetate, altrenogest, norethisterone acetate) can be used to prevent oestrus for as long as administration is continued
- megestrol acetate and medroxyprogesterone acetate are licensed for this purpose
- should the animal enter pro-oestrus during the first few days of treatment, the dosage can be increased
- a period of anoestrus usually follows therapy, so oestrus does not return immediately after cessation of treatment

Oral administration of progestogens during pro-oestrus

- high doses of orally active progestogens (megestrol acetate, medroxyprogesterone acetate, altrenogest, norethisterone acetate) may be given during pro-oestrus to suppress the signs of oestrus

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- megestrol acetate and medroxyprogesterone acetate are licensed for this purpose
- usually the signs of pro-oestrus/oestrus disappear within approximately 5 days
- treatment during late pro-oestrus may not prevent ovulation in the bitch, although conception is unlikely to occur
- treatment too early may lead to a return to oestrus soon after dosing
- a reducing dose regime administered from the first signs of pro-oestrus and continued for up to 16 days is usually efficacious
- this is usually followed by a variable period of anoestrus; oestrus returns between 4 and 6 months after the end of medication

Subcutaneous administration of depot preparations during pro-oestrus

- administration of the new-generation depot progestogens (proligestone) to bitches in early pro-oestrus may be used to suppress the signs of that oestrus
- older-generation progestogens (medroxyprogesterone acetate) which have potent effects upon the uterus are not recommended
- signs of pro-oestrus/oestrus disappear within approximately 5 days
- following the depot progestogen, there is a variable anoestrus, and oestrus returns approximately 6 months later

NB: Other agents that may be used for the control of oestrus cyclicity include long-term administration of androgens commencing in anoestrus.

Treatment of pseudopregnancy (see 3.7 and 19.8)

- administration of progestogens to bitches with clinical signs of pseudopregnancy produces a suppression of prolactin secretion and the clinical signs rapidly disappear
- prolactin concentration is reduced for the duration of progestogen administration
- prolactin may increase again if progestogen therapy is rapidly withdrawn
- progestogens may be administered either orally daily (for example, megestrol acetate) or by depot injection (for example, proligestone or delmadinone acetate)
- first-generation progestogens such as medroxyprogesterone acetate are not recommended because they have marked effects upon the uterus and might potentiate the development of a pyometra
- oral therapy is often associated with relapse of the clinical signs, if therapy is terminated too quickly; this can usually be prevented by gradually reducing the dose over a period of approximately 7 or 10 days
- care must be taken to ensure that the bitch is not pregnant, especially if depot progestogens are to be used because these can delay or prevent parturition
- it is likely that the return to oestrus will be delayed following the administration of progestogens

NB: It is imperative that bitches are confirmed to be pseudopregnant rather than being pregnant (owners may be unaware) since treatment of pregnant bitches can

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delay or prevent parturition resulting in significant illness in bitches [2]. Other options for the treatment of pseudopregnancy include the administration of androgens, oestrogens, or combinations of these, or the use of prolactin antagonists such as cabergoline.

Treatment of habitual abortion (see 21.3)

- there is only anecdotal evidence that habitual abortion occurs in the bitch
- it is therefore not appropriate to supplement bitches with progestogens to prevent habitual abortion
- progestogen supplementation during pregnancy may produce masculinized female pups, cryptorchid male pups, and may possibly impair or delay parturition resulting in fetal death

Clinical use of progestogens in dogs

Antisocial behaviour and other behavioural problems

- aggression, roaming, territory marking, copulatory activity, destruction and excitability exhibited by both entire and castrated dogs may be controlled in some cases by progestogens
- their actions relate both to their anti-androgenic effect and their central sedative action
- depot progestogen therapy may need to be repeated every month for the shorter-acting preparations (delmadinone acetate) and up to every 6 months for the longer-acting preparations (medroxyprogesterone acetate and proligestone)
- oral therapy has also been shown to be effective and has the advantage that the dose may be adjusted to the effect

NB: Behaviour modification training is an essential adjunct to progestogen therapy, and entire dogs should be considered for castration.

Benign prostatic hyperplasia (see 17.7)

- progestogens are both anti-androgenic and have a direct effect upon the prostate gland
- these actions are responsible for the rapid reduction in the clinical signs associated with regression of the prostate gland
- depot therapy usually causes improvement of clinical signs within 7 days, and remission of signs within 14 days, although a second treatment may be necessary in some individuals
- delmadinone acetate is commonly used because of its short period of action and its relative sparing effects upon sperm production
- other depot or orally active progestogens (osaterone acette) are equally efficacious and high doses or long treatment periods are necessary to interfere with fertility; the author has shown that it is therefore possible to titrate the clinical

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benefit of control of prostatic disease without adversely affecting fertility in stud dogs [3]
- in many cases only 1 week of administration is required to produce several months of clinical benefit (e.g. osaterone)
- as well as the negative feedback effects, progestogens also prevent binding of androgens to prostatic receptors which increases their efficacy
- in dogs not required for breeding, castration is the treatment of choice

NB: Other agents that may be used for the treatment of benign prostatic hyperplasia include oestrogens and anti-androgens such as flutamide and finasteride.

Prostatitis

- dogs with bacterial prostatitis require appropriate antibiotic treatment, however control of clinical signs can be speeded by the concomitant administration of progestogens

Prostatic neoplasia (see 17.7)

- some clinical improvement may be seen in dogs with prostatic neoplasia following the administration of progestogens
- the effect is usually only short-term, and progestogens offer little relief in metastatic disease

NB: Other agents that may be used include oestrogens, anti-androgens such as flutamide and finasteride, and gonadotrophin-releasing hormone (GnRH) analogues such as buserelin.

Circumanal adenomata

- the anti-androgenic effect of progestogens given as a depot or oral preparation is useful for causing temporary reduction in the size of these benign tumours
- tumours with central necrosis may not respond well
- in dogs not required for breeding, castration is the treatment of choice

NB: Other agents that may be used include oestrogens.

Contraception

- long-term administration of high doses of progestogens may be useful to cause spermatozoal abnormalities and a possible suppression of spermatogenesis [3]
- the concurrent administration of depot androgens appears to be more efficacious and allows the progestogen dose to be reduced; indeed progestogens are unlikely to be efficacious if used alone
- castration is the method of choice in dogs that are not required for breeding

NB: Other agents that may be used include GnRH agonists.

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27.2 OESTROGENS [1]

- oestrogen is produced by ovarian follicles of the bitch, and the Leydig cells of the dog
- endogenous oestrogens are responsible for the development of the female sexual characteristics
- oestrogens are necessary for normal secretion and functioning of the uterine tubes, and changes in oestrogen concentration regulate the gonadotrophin surge which stimulates ovulation
- oestrogen is involved in the initiation of parturition
- in the male, oestrogens are involved in the feedback control of Leydig cell function
- high doses of exogenous oestrogen cause a negative feedback at the hypothalamic–pituitary axis and subsequent suppression of gonadotrophin secretion
- low doses of oestrogen enhance the release of FSH

Adverse effects of oestrogens

General

- oestrogens have been shown to produce a dose-related bone marrow suppression
- this may result in a severe and possibly fatal anaemia and thrombocytopenia
- prolonged oestrogen therapy may produce a non-pruritic bilaterally symmetrical alopecia and skin hyperpigmentation

Specific adverse effects in the bitch

- oestrogens potentiate the stimulatory effect of progesterone on the uterus, cause cervical relaxation and allow vaginal bacteria to enter the uterus
- oestrogen administration may therefore result in the development of cystic endometrial hyperplasia and pyometra
- large doses of oestrogen may stimulate signs of oestrus in both entire and ovariohysterectomized bitches
- oestrogens may cause cervical relaxation and abortion in the pregnant bitch
- administration during pregnancy may cause congenital defects in the developing fetuses

Specific adverse effects in the dog

- oestrogens may result in abnormalities of semen quality and a resultant reduction in fertility
- prolonged therapy may cause reversible prostatic metaplasia (see 17.7)

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Clinical use of oestrogens in the bitch

Unwanted mating (see 25.7)

- oestrogens alter zygote transport time and impair implantation when administered soon after mating
- oestrogens may also produce a short luteal phase by interfering with luteinizing hormone (LH) support of the corpora lutea
- diethylstilboestrol, oestradiol cypionate and mestranol have been widely used to treat unwanted matings
- only oestradiol benzoate is licensed for the treatment of unwanted mating in the UK
- oestradiol benzoate may be administered at a lower dose 3 and 5 (and possibly also 7) days after mating

NB: Other agents that may be used include aglepristone, the progesterone receptor antagonist. It is also possible to perform surgical neutering or to wait for a diagnosis of pregnancy at 28 days and then to terminate a pregnancy if present with aglepristone or a combination of prolactin inhibitor with prostaglandin.

Pseudopregnancy (see 3.7 and 19.8)

- oestrogens may be used to produce an inhibition of prolactin secretion in bitches with pseudopregnancy
- a preparation of ethinyl oestradiol combined with methyltestosterone was previously available and produced a good clinical response, although the dose suggested seems arbitrary
- recurrence of the clinical signs may follow abrupt termination of treatment

NB: Other treatment options include the administration of progestogens, androgens or specific prolactin antagonists such as cabergoline.

Juvenile vaginitis (see 16.3)

- the condition usually regresses after the first oestrus
- if the clinical signs are severe some control may be effected using low doses of oral oestrogens daily for 5 days
- an alternative therapy is the topical application of oestrogen-containing creams

Oestrus induction (see 24.4)

- low doses of oestrogen enable follicle-stimulating hormone (FSH) to stimulate the formation of LH receptors on granulosa cells
- diethylstilboestrol may be used orally daily for up to 2 days after the onset of pro-oestrus
- ovulation is ensured by the administration of human chorionic gonadotrophin (hCG)

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NB: Other treatment regimes include the administration the prolactin inhibitor cabergoline or equine chorionic gonadotrophin (eCG) followed by human chorionic gonadotrophin.

Urinary incontinence

- in ovariohysterectomized bitches incontinence may relate to changes in the thickness of the urethral mucosa in the absence of oestrogen
- some cases respond to oestrogen therapy, whilst in others the response is short lived or absent

NB: Other agents that may be used include the drugs that act directly upon the urethral muscle such as phenylpropanolamine and depot GnRH agonists.

Clinical use of oestrogens in the dog

Antisocial behaviour and other behavioural problems

- oestrogens may be useful in dogs which show antisocial behaviour
- the potential adverse effects result in progestogens being more commonly used

Benign prostatic hyperplasia (see 17.7)

- repeated administration of oestrogens will result in a reduction in prostatic size and the amelioration of the clinical signs
- the treatment of choice is probably low-dose progestogens which have a minimal effect on fertility, or castration in the animal that is not required for breeding
- prolonged oestrogen therapy may result in prostatic metaplasia and a resultant increase in the size of the prostate gland

NB: Other treatment options include the use of progestogens and GnRH agonists.

Prostatic neoplasia (see 17.7)

- clinical signs of prostatic neoplasia may be controlled in the short term by the administration of oestrogens
- progestogens may also be useful in the short term, whilst in man anti-androgens and GnRH analogues are widely used

Circumanal adenomata

- the anti-androgenic effect of oestrogens may be useful to cause a decrease in the size of these tumours

NB: Other options are the administration of progestogens, although the treatment of choice is castration in dogs that are not required for breeding.

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27.3 ANDROGENS [3]

- the naturally occurring androgens, testosterone and dihydrotestosterone, are produced by the interstitial cells of the testes
- in the male, androgens mediate the development and maintenance of primary and secondary sexual characteristics and normal sexual behaviour and potency
- androgens play an important role in the initiation and maintenance of spermatogenesis
- androgens are generally not present in significant concentrations in the non-pregnant female
- androgens have a negative feedback effect upon the hypothalamic–pituitary axis, and influence, among other things, the release of the gonadotrophins and prolactin
- synthetic androgens have a duration of activity related to the nature of the ester

Adverse effects of androgens

General

- androgen therapy may produce virilizing effects such as aggression, and their use may be contra-indicated in dogs with existing behavioural problems
- in pre-pubertal animals premature epiphyseal growth plate closure may occur
- androgens are contra-indicated in nephrotic conditions because the anabolic component causes both sodium and water retention

Specific adverse effects in the bitch

- prolonged androgen administration may result in clitoral hypertrophy, and very rarely the development of an os clitoris
- repeated or prolonged therapy may result in a persistent vaginitis
- severe urogenital abnormalities may develop in female fetuses if androgens are administered to bitches during pregnancy (see Chapter 19)

Specific adverse effects in the dog

- high doses of androgen produce severe changes in spermatozoal morphology and fertility

Clinical use of androgens in the bitch

Control of oestrus (see Chapter 25)

- androgens may be administered to bitches in anoestrus to prevent a return to cyclical activity
- androgens are not useful for inhibiting oestrus in females with follicular activity (pro-oestrus or early oestrus)

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- administration must commence in late anoestrus, at least 30 days before the onset of pro-oestrus
- they do not mimic the luteal phase, and there is no subsequent anoestrus; therefore cyclical activity returns rapidly after the cessation of treatment
- in the UK the most common regime is the administration of depot androgens during anoestrus either as a prolonged-release implant, or depot injection of mixed testosterone esters; it is not uncommon for depot therapy to be supplemented by daily oral therapy
- in other countries an orally active synthetic androgen, mibolerone, is available

NB: Other agents that may be used to control oestrus include the progestogens.

Pseudopregnancy (see 3.7 and 19.8)

- relatively high doses of androgens may be used to produce an inhibition of prolactin secretion
- this results in a rapid resolution of the clinical signs
- androgens may be more useful than either progestogens or oestrogens because they do not have any adverse effects on the uterus
- oral methyltestosterone or parenteral testosterone esters are efficacious; however, the orally active methyltestosterone combined with ethinyl oestradiol was previously available which produced a good clinical response

NB: Other treatment options for pseudopregnancy include the administration of progestogens, oestrogens alone, or specific prolactin inhibitors such as cabergoline.

Clinical use of androgens in the dog

Poor libido (see 20.2)

- there is no evidence in the dog that poor libido is caused by low circulating androgen concentrations
- androgens should not be used in these cases because they produce significant changes in spermatozoal morphology and fertility [3]

NB: Other agents that may be useful in dogs with poor libido include hCG, which produces an increase in endogenous testosterone concentration.

Poor semen quality (see 17.3 and 20.3)

- androgen supplementation is commonly used in these cases, and this may produce disastrous results because the androgens suppress spermatogenesis via the negative feedback mechanism

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- in the author's laboratory we demonstrated a substantial effect of androgens on semen quality which resulted in azoospermia within a few weeks [3]
- an analogue of dihydrotestosterone, mesterolone, is available in the UK for human use and is unusual in that it is not aromatized to oestradiol and does not significantly suppress the release of pituitary gonadotrophins; in the author's clinic the drug was shown to have a positive effect on semen quality of some cases [4]

Contraception

- long-term administration of high doses of androgens may be useful for reducing semen quality
- the concurrent administration of progestogens appears to be more efficacious [3]

Cryptorchidism (see 17.3)

- medical therapy with androgens has not been shown to be efficacious and is unethical

27.4 GONADOTROPHINS

- the gonadotrophins FSH and LH are secreted by the anterior pituitary gland
- in the bitch, FSH stimulates initial follicular development and its surge release is associated with the process of ovulation
- in the bitch, LH also stimulates follicular growth, is the trigger for ovulation and is a principal luteotrophic agent
- in the dog FSH stimulates spermatogenesis indirectly by an action upon the Sertoli cells, and LH stimulates the Leydig cells to produce testosterone
- neither FSH or LH are available for use in the dog; however
  (a) equine chorionic gonadotrophin (eCG) is mainly FSH-like in activity
  (b) human chorionic gonadotrophin (hCG) is primarily LH-like in effect

Adverse effects of gonadotrophins

General

- there is a risk of inducing anaphylactoid reactions and antibody formation following the injection of these protein preparations

Specific adverse effects in the bitch

- hCG if given to oestrous bitches may cause luteinization of follicles and interfere with ovulation
- hyperstimulation of the ovary by the administration of high doses of eCG or the use of repeated therapy may result in non-ovulating follicles and persistent oestrus behaviour

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- a persistent elevation in plasma oestrogen concentration may lead to adverse effects typical of oestrogen toxicity

Specific adverse effects in the dog

- none

Clinical use of gonadotrophins in the bitch

Induction of oestrus (see 24.4)

- repeated doses of eCG given to anoestrous bitches stimulate follicular growth and the production of oestrogen
- ovulation may occur spontaneously, following an endogenous surge of LH, or may be induced using hCG
- care must be observed when using eCG and hCG combinations because endogenous hyperoestrogenism may occur and result in inhibition of implantation, bone marrow suppression and death
- low doses of gonadotrophins are suggested to produce more physiological plasma oestrogen profiles
- a relatively low plasma progesterone concentration or short luteal phase following gonadotrophin-induced oestrus is common
- in the author's clinic we demonstrated the value of gonadotrophin regimes for oestrus induction of bitches [5]

NB: Other agents that may be useful for the induction of oestrus include low-dose oestrogens, or prolactin inhibitors such as cabergoline.

Delayed puberty (see 19.1)

- therapy to induce oestrus may be attempted using an eCG/hCG regime

Hastening of ovulation (see 19.4)

- bitches that repeatedly fail to conceive are sometimes given hCG at the time of mating on the assumption that ovulation has not occurred or that early development of the corpora lutea is inadequate; there is no evidence that this is the case, and such 'blind' therapy cannot be recommended
- some bitches may have prolonged pro-oestrus or oestrus and the administration of hCG may possibly hasten ovulation; hCG is administered when more than 90% of vaginal epithelial cells are anuclear, or when a slight rise in plasma progesterone concentration has been detected
- premature administration of hCG may result in follicular luteinization and failure of ovulation

NB: GnRH may be used in a similar manner to hasten ovulation.

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Identification of ovarian tissue

- it may be difficult to determine clinically whether a bitch has been ovariohysterectomized
- the administration of hCG to bitches with ovaries results in an increase in plasma oestrogen concentration
- this method may therefore be clinically useful for detecting the presence of ovaries

NB: The administration of GnRH may also be clinically useful for the identification of spayed bitches.

Clinical use of gonadotrophins in the dog

Diagnosis of testicular tissue (see 17.3)

- in male dogs with no scrotal testes the presence of testicular tissue can be confirmed by performing an hCG stimulation test (see 10.15)
- plasma testosterone concentration is measured before and 60 minutes after the intravenous administration of hCG; a significant rise in testosterone concentration is diagnostic of testicular tissue

Cryptorchidism (see 17.3)

- testicular descent will not occur following the administration of gonadotrophins
- the condition is likely to be inherited and medical treatment is unethical

Hypogonadism (see 20.1)

- treatment with gonadotrophins is not successful

Poor libido (see 20.2)

- a transient increase in libido may follow the administration of hCG to male dogs
- this effect is the result of increased endogenous testosterone concentrations

27.5 GONADOTROPHIN-RELEASING HORMONE (GnRH) AGONISTS

- GnRH controls gonadotrophin synthesis via a neuroendocrine mechanism
- several synthetic GnRH agonists are available, the one licensed most widely for use in dogs is deslorelin which is described as a 'super-agonist'
  (a) GnRH agonists cause an increase in the production of FSH and LH
  (b) however, their repeated or depot administration results in a down-regulation of the receptors thus preventing the release of FSH and LH (in essence then working like an antagonist)

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- GnRH antagonists produce a reduction in circulating gonadotrophins within a few hours but no commercially available products are available for use in animals (several are available as human medicinal products for the treatment of prostatic neoplasia; they are largely untested in clinical situations in dogs)

Adverse effects of GnRH agonists

General

- no general adverse effects have been reported with these agents in dogs

Specific adverse effects in the bitch

- repeated or depot administration of GnRH agonists may result in absence of oestrus (of course this is a reason for use in some circumstances)
- bitches in which oestrus is induced with GnRH agonists may have a short luteal phase which results in pregnancy loss

Specific adverse effects in the dog

- repeated or depot administration of GnRH agonists may cause a reduction in libido, and long-term administration may cause reduced spermatozoal morphology and fertility (of course this is a reason for use in some circumstances)
- in a small proportion of dogs there are changes in coat colour and hair loss

Clinical use of GnRH agonists in the bitch

Identification of ovarian tissue

- the administration of a short acting GnRH (buserelin) to intact bitches produces an increase in LH and subsequent rise in plasma oestrogen concentration
- this test may be useful for the detection of bitches that have been ovariectomized or ovariohysterectomized because no rise in oestrogen occurs

NB: The GnRH stimulation test requires the pituitary gland to also be functional. An alternative method is to administer hCG and monitor plasma oestrogen concentration, which increases in bitches with ovarian tissue.

Hastening of ovulation (see 19.4)

- single doses of a short acting GnRH agonist may be useful to hasten ovulation in bitches that are in oestrus
- the use of hCG, however, may be more efficacious, although neither drug has been adequately evaluated for this purpose

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Induction of oestrus (see 24.4)

- placement of a GnRH agonist implant in anoestus bitches will cause increased secretion of FSH and LH and result in the onset of pro-oestrus
- if the implant is removed soon after signs of pro-oestrus are seen, the down-regulation response is avoided and pro-oestrus and oestrus progress
- induced cycles have good fertility but some bitches have a short luteal phase resulting in pregnancy loss

NB: Other agents that can be used for oestrus induction include prolactin inhibitors.

Control of oestrus (see 25.5)

- placement of a GnRH agonist implant in anoestrus bitches will result in receptor down-regulation and prevent cyclical activity
- initial stimulation and signs of pro-oestrus will occur in many bitches shortly (approximately 4 days) after implantation
- placement of further implants approximately every 5 months will result in permanent contraception
- bitches return to reasonable fertility after cessation of implantation, although the time to return to cyclical activity is not predictable
- it is feasible to place the implant in the luteal phase and fewer bitches show signs of pro-oestrus, however it is possible that uterine disease may occur in these cases (as oestrogen concentration increases when there is already elevated progesterone) so this regime is not recommended

NB: Other agents that may be used to control oestrus include progestogens and androgens.

Unwanted mating (see 25.7)

- the down-regulation effects of continuous GnRH administration may be used in attempt to withdraw gonadotrophin support of the corpus luteum
- suitable clinical trials have, however, not been performed to confirm efficacy

NB: Other agents that may be used to induce resorption or abortion include oestrogens, prostaglandins, prolactin antagonists and progesterone antagonists.

Urinary incontinence

- bitches (and a small number of dogs) develop an overflow urinary incontinence after surgical neutering
- these are often described as sphincter mechanism incontinence and are thought to be due to low concentrations of plasma oestrogen
- however as well as low oestrogen concentrations these animals have high concentrations of LH and FSH because there are no gonadal steroids producing a negative feedback effect

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- LH, FSH and GnRH receptors are present on urethral smooth muscle and administration of GnRH agonists has been shown to produce a resolution of incontinence in some cases

NB: Other agents with greater efficacy are oestrogen and alpha agonists such as phenylpropanolamine.

Clinical use of GnRH agonists in the dog

Contraception and control of male behaviour

- placement of GnRH agonist implants may be useful for the suppression of LH and FSH, causing a reduced concentration of testosterone and therefore inhibited libido and infertility
- this effect appears to be a useful method of inducing a reversible contraception with an effect within 6 weeks of implantation lasting for approximately 6 months (there is considerable variation but most dogs have normal testosterone concentrations 12 months after implantation)
- use of an implant can be for a temporary control, to establish what the likely effect on castration on behaviour will be, or where the owner wishes to avoid surgery

NB: In some dogs behaviour is learned and does not respond well to hormonal intervention. Other products such as progestogens are useful for control of behaviour. Other products such as progestogens, androgens or progestogen-androgen combinations are suitable for controlling fertility. Castration is the method of choice in the animal that is not required for breeding.

Prostatic disease (see 17.7)

- repeated or depot administration of GnRH agonists may be useful in dogs which have benign prostatic hyperplasia and prostatic neoplasia

27.6 GONADOTROPHIN-RELEASING HORMONE (GnRH) ANTAGONISTS

- few GnRH antagonists have found clinical use in small animal reproduction because of adverse effects of the formulations and short duration of action necessitating repeated administration
- recently the experimental use of acyline, which has high receptor affinity and reasonable duration of action after a single administration, has been to be useful for termination of pregnancy at approximately day 30
- there is no doubt that such a product would find wide clinical use in controlling reproduction in males and females if it were to be commercially available

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27.7 PROSTAGLANDINS

- the reproductive prostaglandins are synthesized in the endometrium, and are both luteolytic and spasmogenic in nature
- prostaglandins are involved in the termination of the luteal phase and the initiation of parturition
- there are no naturally occurring or synthetic prostaglandin analogues licensed for use in the bitch
- the naturally occurring prostaglandin F2alpha, dinoprost and several synthetic prostaglandin analogues (especially cloprostenol and luprostiol) have, however, been widely used
- when prostaglandins are required principally for luteolysis, the synthetic prostaglandins should be used (e.g. cloprostenol)
- when prostaglandins are required principally of uterine spasmogenic actions, synthetic naturally occurring prostaglandins should be used (e.g. dinoprost)

Adverse effects of prostaglandins

General

- prostaglandin administration may be followed by restlessness, hypersalivation, vomiting, abdominal pain, pyrexia, tachycardia, ataxia and diarrhoea
- high doses may be lethal
- these effects are entirely dose-related and with low-dose regimes very few effects are seen
- adverse effects develop quickly after administration and usually persist for up to 60 minutes

Specific adverse effects in the bitch

- cases of closed-cervix pyometra given prostaglandin may develop uterine rupture

Specific adverse effects in the dog

- prostaglandins are not used in male dogs

Clinical use of prostaglandins in the bitch

Termination of pregnancy (see 25.7)

- low doses of dinoprost or cloprostenol given daily, or twice daily, for several days can be used to produce luteal regression
- repeated low doses cause lysis of the corpora lutea and induce abortion, especially when given later than 23 days after ovulation

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- earlier treatment may not be efficacious because developing corpora lutea are more resistant to the effects of prostaglandin
- a better therapeutic option is to use a combination of prostaglandin and a prolactin inhibitor such as cabergoline

Treatment of pyometra (see 16.5)

- prostaglandins may be used to induce uterine emptying (the result of their spasmogenic activity) and to remove the stimulatory effects of progesterone (the result of their luteolytic action)
- dinoprost administered at low doses twice daily for 5 days, combined with appropriate antibiotic and fluid therapy, may be used as a successful treatment
- following treatment approximately 20% of bitches return to fertility
- the long-term complications include recurrence of pyometra, anoestrus, failure to conceive and abortion
- a better therapeutic option is to use a combination of prostaglandin and a prolactin inhibitor such as cabergoline

NB: Other agents that may be used to treat cases of pyometra include progesterone receptor antagonists such as aglepristone.

Post-partum metritis (see 22.12)

- post-partum metritis in the bitch may be treated by using prostaglandins to induce uterine evacuation
- antimicrobial preparations are mandatory; however, in these cases there is no underlying hormonal component (plasma progesterone concentration is low) and therefore a rapid resolution should be expected
- a regime similar to that used for the treatment of pyometra may be employed

NB: Other treatment options include the administration of oxytocin or ergometrine.

Induction of oestrus (see 24.4)

- prostaglandins may be used to induce luteolysis; however, the luteal phase is followed by a variable period of anoestrus
- shortening of the luteal phase does not therefore produce a rapid return to oestrus

NB: Methods of oestrus induction include the use of prolactin inhibitors and GnRH agonists.

Clinical use of prostaglandins in the dog

- there are no therapeutic roles for reproductive prostaglandins in the dog

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27.8 PROGESTERONE ANTAGONISTS

- specific progesterone receptor antagonists have antiprogestogenic effects
- the availability of these agents varies from one country to another
- aglepristone is available in many countries and is available as an injectable solution
- mifepristone (RU486) is available as an orally active product, but sale is limited in some countries because of potential human misuse
- these products have a high affinity for the progesterone receptor and will displace progesterone after administration; there is therefore no biological signal although there is no initial change in plasma progesterone concentration

Adverse effects of progesterone antagonists

General

- subcutaneous administration of aglepristone is associated with an incidence of injection site reaction; for large breeds of dog several injections sites are used to reduce the risk of problems

Specific adverse effects in the bitch

- administration of progesterone antagonists to pregnant bitches will result in resorption, abortion or premature parturition, depending upon the time of administration

Specific adverse effects in the dog

- there are no therapeutic uses of progesterone antagonists in male dogs

Clinical use of progesterone antagonists in the bitch

Pregnancy termination (see 25.7)

- since aglepristone prevents any biological signal from progesterone it can be used to prevent implantation or to terminate pregnancy at any stage
- two doses of aglepristone are given 24 hours apart and this results in elevated concentrations for several days
- when given to bitches in oestrus the long duration of action will have an effect upon progesterone when it is released thus blocking progesterone action and preventing implantation
- when given in early pregnancy resorption will follow
- when given in mid pregnancy abortion will follow
- when given in late pregnancy fetal death will occur but there may not be expulsion and additional treatment with prostaglandin may be necessary

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Pyometra (see 16.5)

- aglepristone administration on days 1, 3, 5, 8 and 16 after presentation appears to be successful for the treatment of pyometra in the majority of dogs
- the use so far has been limited; however, the regime produced no adverse effects, and emptying of uterine fluid occurred quickly producing resolution of the clinical signs
- some of the bitches returned to normal fertility after treatment
- improved outcomes can be achieved with the concomitant administration of prostaglandin

Induction of parturition

- there are few indications for induction of parturition in bitches; a reliable technique might be useful for management reasons enabling effective supervision of whelping, or for management of cases of acute illness in the dam
- induction of parturition can be achieved by the administration of two doses of aglepristone followed 24 hours later by repeated injection of oxytocin; the dose and frequency of oxytocin is lower the closer the bitch is to spontaneous parturition

Clinical use of progesterone antagonists in the dog

- there are no clinical indications for the use of progesterone antagonists in male dogs

27.9 PROLACTIN INHIBITORS [6]

- prolactin is the primary luteotrophic factor of the pregnant and the non-pregnant luteal phase
- initially corpora lutea appear to be autonomous and do not require other hormonal support
- however after day 20 onwards prolactin concentrations increase and prolactin becomes the most important support for the corpora lutea; prolactin concentrations are then maintained for the duration of the luteal phase
- prolactin inhibitors cause a rapid decrease in prolactin concentration and a subsequent decline in plasma progesterone concentration (Figure 27.1)
- prolonged prolactin suppression results in a termination of the luteal phase, and the bitch enters anoestrus
- continual administration of prolactin antagonists during anoestrus causes a return to oestrus sooner than anticipated; the mechanism of this oestrus-induction action is unknown
- bromocriptine and cabergoline are ergot derivatives that inhibit prolactin release by direct stimulation of dopaminergic receptors of prolactin-releasing cells in the anterior pituitary gland

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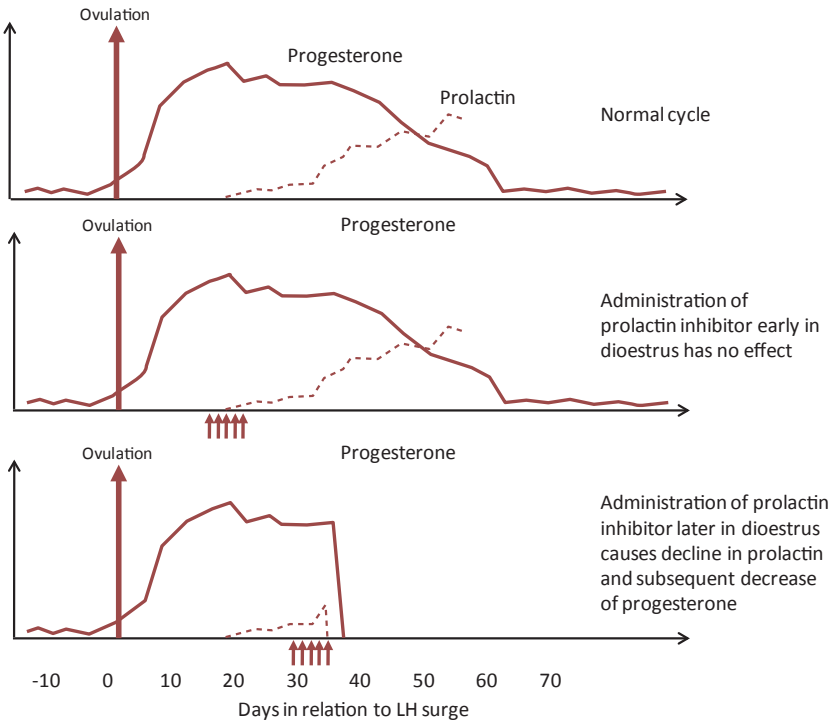


Figure 27.1 Schematic demonstration of the luteotrophic role of prolactin and the effect of administration of prolactin inhibitor.

- cabergoline appears to be a more specific agent that has fewer side-effects attributable to dopaminergic stimulation of the central nervous system
- cabergoline is widely licensed for use in the dog

Adverse effects of prolactin inhibitors

General

- dopaminergic stimulation may result in nausea and vomiting especially with the first-generation product, bromocriptine
- lethargy and occasional constipation may also be noticed
- the adverse effects of bromocriptine can be reduced by using the minimal effective dose and mixing the drug with food. Specific anti-emetics such as metoclopramide may prevent vomiting, but whilst they are clinically useful, their administration does not make pharmacological sense because they also work via dopamine receptors
- all adverse effects are uncommon following cabergoline administration

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Specific adverse effects in the bitch

- administration of prolactin antagonists during pregnancy may cause abortion
- repeated administration will result in a shortening of anoestrus and a return to oestrus

Specific adverse effects in the dog

- there are no clinical applications of prolactin antagonists in male dogs

Clinical use of prolactin inhibitors in the bitch

Pseudopregnancy (see 3.7 and 19.8)

- suppression of prolactin causes a rapid resolution of the clinical signs of pseudopregnancy
- cabergoline is widely available and had good clinical acceptability; occasional vomiting is seen but this can be reduced by mixing the drug with food, or the prior administration of metoclopramide may also be useful as discussed above
- usually clinical signs regress quickly and treatment is only required for 5 or 7 days

NB: Other agents that may be used for the treatment of pseudopregnancy include progestogens, androgens, oestrogens and androgen–oestrogen combinations.

Iatrogenic or persistent psuedopregnancy

- some iatrogenic cases of pseudopregnancy (induced by neutering during the mid and late luteal phase) are persistent; these and persistent spontaneous cases can be treated by prolonged administration of prolactin inhibitors
- treatment may be required for up to 3 weeks

Galactostasis (see 22.21)

- excessive milk accumulation within the mammary gland seen especially at the time of weaning can be managed by a short-duration course of cabergoline

Treatment of pyometra

- repeated administration of prolactin inhibitors will reduce progesterone and cause opening of the cervix as well as allowing increased uterine contractions
- the concomitant administration of prostaglandin also causes uterine contraction and lysis of the corpora lutea
- these synergistic actions can be useful in the treatment of pyometra
- for closed-cervix pyometra, as long as the prolactin inhibitor is administered for several days before the prostaglandin, the cervix will open and the risk of uterine rupture is low compared with regimes using prostaglandin alone

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- in the author's clinic we have demonstrated high clinical efficacy using 5.0 µg/kg cabergoline daily for 10 days with 5.0 µg/kg cloprostenol administered on days 3,6 and 9 [7]
- this regime is now widely used in clinical practice

Termination of pregnancy (see 25.7)

- repeated administration of the prolactin inhibitors produces a reduction in plasma progesterone concentration and resorption or abortion
- efficacy can be increased, and treatment may be given earlier, when prolactin antagonists and prostaglandins are given simultaneously
- both agents are more effective when given later in pregnancy because the role of prolactin is more important at this time
- common regimes involve the 10-day administration of prolactin inhibitors with prostaglandin being injected every second day on five occasions

NB: Other agents that may be used to terminate pregnancy include prostaglandins or progesterone antagonists.

Induction of oestrus (see 24.4)

- apparently normal and fertile oestrous periods may be induced in anoestrous bitches following continual administration of bromocriptine or cabergoline
- the time to the onset of oestrus seems to relate to the stage of anoestrus; bitches in late anoestrus respond more quickly than those in early anoestrus. Treatment starting in the luteal phase may also be effective but the treatment period is longer
- the induced oestrus appears to be physiological, and whilst the mechanism of action is uncertain it may be the result of inhibition of remnant progesterone production by the corpora lutea

NB: Other agents that may be used to induce oestrus include low-dose oestrogens or gonadotrophin combinations.

Clinical use of prolactin inhibitors in the dog

- there are no therapeutic roles for prolactin antagonists in the dog

27.10 OXYTOCIN AND ERGOT PREPARATIONS

- oxytocin receptors develop on the myoepithelial cells of the mammary gland shortly before parturition
- uterine oxytocin receptors also increase in number close to parturition, and oxytocin release stimulates contraction of the oestrogen-primed uterus

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- several formulations of oxytocin and posterior pituitary extract are available commercially, and can be used to stimulate the smooth muscle of the mammary and genital tract
- ergotamines are alkaloid extracts of a rye fungus which are ecbolic agents; they produce a prolonged spasm of uterine muscle with relaxation only after 1 or 2 hours when the uterus then contracts rhythmically in a similar manner to that produced by oxytocin

Adverse effects of oxytocin

General

- there is a low incidence of skin sloughing or abscess formation recorded by some manufacturers following the subcutaneous administration of oxytocin
- ergot alkaloids may produce emesis and slight stimulation of the central nervous system

Specific adverse effects in the bitch

- oxytocin administration to a bitch in late pregnancy may initiate premature parturition

Specific adverse effects in the dog

- oxytocin and ergot preparations have no clinical indications in the dog

Clinical use of oxytocin in the bitch

Uterine inertia (see 22.1)

- cases of primary uterine inertia or secondary uterine inertia (following correction of the dystocia) may respond to parenteral oxytocin administration
- oxytocin has a short half-life and therefore repeated administration (every 15–30 minutes) may be necessary to maintain a clinical effect
- the use of a continuous low-dose intravenous infusion does not appear to have been investigated in bitches
- oxytocin is contra-indicated in obstructive dystocia because uterine rupture may result
- ergot preparations may also be used in cases of inertia; however, they produce prolonged uterine contractions and are less suitable

Placental retention or fetal retention (see 22.11)

- oxytocin is usually effective at producing uterine contraction and expulsion of the pup and/or placenta

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- the response to oxytocin rapidly decreases after parturition, and in cases that are presented late, a hysterotomy may be necessary
- ergot preparations may be used in these cases but are less efficacious because of the prolonged contractions that they produce

Post-partum haemorrhage (see 22.13)

- persistent uterine haemorrhage may be controlled by oxytocin administration (vaginal haemorrhage is best controlled by use of a tampon)
- ergometrine may be a more useful agent in cases of uterine haemorrhage because of the initial prolonged uterine contractions
- excessive haemorrhage, although very rare, may necessitate a hysterotomy to identify and correct the cause

Post-partum metritis (see 22.12)

- uterine evacuation may be induced by oxytocin administration in cases of post-partum metritis however uterine oxytocin receptors decrease after parturition such that treatment efficacy reduces each day after parturition
- appropriate antibiotic therapy is mandatory

NB: Other treatment options include the administration of prostaglandins or ergometrine and probably either of these are more appropriate than oxytocin.

Subinvolution of placental sites (see 22.14)

- most cases regress spontaneously after the bitch has returned to oestrus
- oxytocin has little effect once the clinical signs are established, but some anecdotal reports suggest that treatment at the time of parturition may prevent the condition from developing

Agalactia

- lack of milk production is rare in the bitch; drugs that stimulate prolactin (including metoclopramide) may be useful
- an absence of milk letdown is relatively common; it can be treated by the administration of oxytocin
- oxytocin does not increase milk production

Clinical use of oxytocin in the dog

- there are no clinical applications for oxytocin therapy in male dogs

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27.11 SYNTHETIC ANTI-ANDROGENS

- anti-androgens may have several mechanisms of action:
  (a) osaterone and cyproterone are progestogens which are principally anti-androgenic
  (b) flutamide inhibits androgen uptake and/or nuclear binding of androgen
  (c) finasteride is a specific 5-alpha reductase inhibitor which prevents the conversion of testosterone into dihydrotestosterone
  (d) formestane is an aromatase inhibitor which inhibits the conversion of androgen to oestrogen in peripheral tissue

Adverse effects of synthetic anti-androgens

General

- adverse effects of these agents have not been reported in dogs, but in man include nausea, gastrointestinal upsets and gynaecomastia

Specific adverse effects in the bitch

- these agents have no clinical indication in the bitch

Specific adverse effects in the dog

- prolonged anti-androgen administration may result in a reduction in semen quality and poor fertility

Clinical use of synthetic anti-androgens in the bitch

- there are no clinical indications for the use of these products in the bitch

Clinical use of synthetic anti-androgens in the dog

Benign prostatic hyperplasia (see 17.7)

- dogs with prostatic hyperplasia may respond to the administration of anti-androgens by a reduction in prostatic size and disappearance of the clinical signs
- the concern with the use of these products is that there may also be a reduction in fertility
- flutamide has been shown to reduce prostatic size with no change in libido or sperm output

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- finasteride has been shown to produce a dramatic decrease in prostatic size and a reduction in the secretion of prostatic fluid; however, total spermatozoal output did not change and fertility remained unaltered
- formestane has not been evaluated for use in dogs

NB: Other preparations that may be used to treat benign prostatic hyperplasia include progestogens and oestrogens.

Prostatic neoplasia (see 17.7)

- anti-androgens have not been reported for use in cases of prostatic neoplasia; however, flutamide, finasteride and formestane have been shown to be useful for the control of clinical signs in man

REFERENCES

1 England, G.C.W. (1994) Hormonal manipulation of breeding in the bitch. *The Veterinary Annual* **34**, 189–200.
2 England, G.C.W (1998) Complications of treating presumed pseudopregnancy in pregnant bitches. *Veterinary Record* **142**, 369–371.
3 England, G.C.W. (1997) Effect of progestogens and androgens upon spermatogenesis and steroidogenesis in dogs. *Journal of Reproduction and Fertility* **51**(Supplement), 123–138.
4 England, G.C.W. and Allen, W.E. (1991) The effect of the synthetic androgen mesterolone upon seminal characteristics of dogs. *Journal of Small Animal Practice* **32**, 271–274.
5 England, G.C.W. and Allen, W.E. (1991) Repeatability of events during spontaneous and gonadotrophin-induced oestrus in bitches. *Journal of Reproduction and Fertility* **93**, 443–448.
6 Verstegen, J.P. and England, G.C.W. (1999) Developments in the control of reproduction in the bitch. *Reproduction in Domestic Animals* **34**, 381–384.
7 England, G.C.W., Freeman, S.L. and Russo, M. (2007) Treatment of spontaneous pyometra in the bitch with a combination of cabergoline and prostaglandin. *Veterinary Record* **160**, 293–296.

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28 Reproductive Biotechnology

There have been substantial advances in reproductive biotechnology over the last 50 years, commencing with artificial insemination with fresh semen, followed by cryopreservation of semen and now new reproductive technologies are widely used in many veterinary species and some are now being introduced for use in the dog and bitch.

28.1 ARTIFICIAL INSEMINATION

Principles of artificial insemination

Artificial insemination (AI) is the technique of collecting semen from a male animal, and placing it into the reproductive tract of the female.

- AI may involve the use of:
  (a) freshly collected semen
  (b) semen that has been diluted and chilled
  (c) semen that has been frozen and thawed
- AI is widely used throughout the world
- in some countries there is limited use of AI because of the restrictions imposed by the national registering authorities
- there is, however, increasing interest in frozen semen inseminations worldwide
- before AI is undertaken a semen sample should be collected from the dog to ensure it is of suitable quality, and to familiarize the dog with the procedure

Advantages of artificial insemination

- AI reduces the requirement to transport animals
- it overcomes the quarantine restrictions that prevent the movement of animals from one country to another
- it increases the genetic pool available to an individual breed within a country
- it reduces the disease risk when unknown animals enter a kennel for mating

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- in some countries the use of AI may reduce the spread of infectious diseases
- in certain circumstances, artificial insemination may be useful when natural mating is difficult, for example bitches which ovulate when they are not in standing oestrus or bitches that have hyperplasia of the vaginal floor
- semen may be collected from male animals which due to age, debility, back pain or premature ejaculation are unable to achieve a natural mating
- the greatest area of interest is probably the storage of genetic material by freezing semen for insemination at a future date

Disdvantages of artificial insemination

- collection of semen from some dogs may be difficult
- theoretically a dog could become 'overused' within a population
- semen from some dogs is not suitable for chilling or freeze–thawing
- there is the potential for error in sample identification
- semen that is cryopreserved has poorer quality and lower fertility than fresh semen; in the author's laboratory we have shown that much of this is associated with ultra-structural changes of the sperm [1], and this knowledge may assist in future studies designed to improve methods for sperm preservation
- there are important ethical dilemmas, especially concerning surgical techniques of artificial insemination, and they are frequently not considered [2]

Regulation concerning the use of artificial insemination

- in some countries pups which are the result of AI can only be registered if the national registering authority has given prior permission
- specific regulations are set regarding the import and export of semen by national veterinary health organizations
- specific regulations can change quickly depending upon national disease status and these should always be checked before embarking on semen transportation (e.g. regulations may alter if there is a change in national status relating to poultry since many semen extenders contain egg yolk)
- additional regulations govern the transportation of frozen semen which is shipped in liquid nitrogen (a dangerous cargo)
- during shipping semen may be inspected by radiographic (x-ray) examination; in the author's laboratory we have shown that this does not have a detrimental effect on semen quality or fertility [3]

Methods of use of semen

- in the author's laboratory we have demonstrated a significant deleterious impact on semen quality of a number of the procedures and practices used in semen preservation [4–5]; this information is important since avoidance of these has had a significant impact upon clinical application of this technology to dogs

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NB: It is perfectly feasible to collect several semen samples within 1 day from a normal dog [6].

Fresh semen

- may be used diluted or undiluted
- may be used when there is inability to mate the bitch (e.g. aggressive bitch, unwilling dog, injury)

Chilled semen

- semen may be stored for a short period of time by chilling and re-warming:
  (a) this allows transportation of semen
  (b) this may be useful when there is short-term unavailability of the dog

Frozen semen

- semen may be stored for long periods of time by freezing and thawing:
  (a) this allows transportation of semen
  (b) genetic material may be stored for a long period of time

Pathogens that may be spread in dog semen

- little information is known about transmission of infectious organisms in semen:
  (a) canine herpes virus may be transmitted at coitus, but it seems likely that this is via direct contact rather than within semen
  (b) rabies virus is unlikely to be found in semen except possibly during a few days before overt clinical signs of the disease
  (c) canine transmissible venereal tumour may be transmitted at coitus, probably by contact and not in semen

Semen preservation

- semen may be preserved by the following methods:
  (a) fresh and cooled to room temperature
  (b) diluted and cooled to 5 °C
  (c) diluted and frozen (–196 °C) then thawed
- the lifespan of preserved semen depends upon the preservation method [7]:
  (a) fresh semen: 4 hours (although this may be lengthened by initial dilution)
  (b) diluted and cooled semen: 4 days
  (c) diluted and frozen semen: indefinite
- semen is normally diluted in an 'extender solution' which aims to:
  (a) protect spermatozoa during cooling/freezing/warming
  (b) supply an energy source to spermatozoa
  (c) maintain pH, osmolarity and ionic strength

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- for many species the optimal pH for survival is approximately 7.0. During storage hydrogen ions are produced by spermatozoa therefore the pH falls. Control of pH is important and many agents have been used:
  (a) phosphate buffer
  (b) egg yolk
  (c) milk proteins
  (d) zwitterionic buffers, e.g. Tris, Tes, Hepes
- hypotonic diluents are harmful because they lead to a gain in intracellular water and redistribution of ions; hypertonic diluents are less harmful because they lead to water loss and a reduction in the likelihood of intracellular ice crystallization. Seminal plasma is 300 mOsm; thus most diluents are approximately 370 mOsm
- glucose, fructose and mannose are glycolysable sugars that may be included in semen extenders as sources of energy
- the larger molecular weight sugars (ribose, arabinose) are often used as non-penetrating cryoproctectants
- antibacterial agents are usually included in semen extenders to control proliferation of microorganisms
- specific protective agents may be used including:
  (a) milk proteins which protect against cold shock
  (b) egg yolk (low density lipoproteins) and bovine serum albumen which protect acrosomal and mitochondrial membranes, and protect against cold shock
- cryoprotective agents are essential for semen freezing because they prevent ice crystal damage; they may be divided into those that are:
  (a) penetrating, (e.g. glycerol and dimethyl sulphoxide [DMSO])
  (b) non-penetrating, (e.g. sugars and polyvinyl pyrollidone)

Preparation of fresh dog semen

- a semen sample is collected as previously described (see 10.5)
- the second fraction is evaluated and used for insemination
- one ejaculate is used for each insemination
- once collected, semen may be deposited into the vagina of the bitch using a long inseminating pipette which is gently introduced near to the cervix (success will likely be increased if semen is placed into the uterus but vaginal deposition gives adequate pregnancy rates)
- when semen is placed in this position spermatozoa must swim through the cervix, into the uterus and up the uterine horns

Preparation of chilled dog semen

- a common extender is composed of non-fat dry skimmed milk, 2.4 g; glucose, 4.9 g; sodium bicarbonate, 0.15 g; sufficient de-ionized water to make the

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volume up to 100 ml. Antimicrobial agents may then be added: penicillin 150 000 IU, and streptomycin 150 000 mg
- alternatively a low-fat pasteurized cow's milk extender can be prepared by heating the milk until it starts to simmer, and then allowing it to cool to 37 °C
- the extender is kept at 37 °C in a water bath immediately before use
- the semen is collected and evaluated
- a small portion of semen is mixed with a small volume of the extender and then evaluated
- providing that the extender has no deleterious effect, the remaining semen is diluted with the extender at 37 °C
- it is preferable to dilute the semen in a semen:extender ratio of 1:4
- the extended semen is cooled slowly to 5 °C and stored at this temperature
- slow cooling can be achieved by placing the extended semen in an insulated vial which is then put in a refrigerator
- semen may be transported before being inseminated
- transportation can be easily achieved using a wide-mouthed thermos flask partially filled with ice cubes
- it is important to perform a trial storage before shipping semen
- semen is normally warmed slowly by placing the vials in a water bath at 37 °C
- uterine insemination is best with semen that has been chilled and vaginal deposition is not recommended

Preparation of frozen–thawed dog semen [8]

- an extender containing: Tris, 6.06 g; fructose, 2.5 g; citric acid, 3.4 g; de-ionized water, 184 ml is used. To this is added glycerol and egg yolk to a final concentration of 8% and 20%, respectively. Often penicillin and streptomycin are added to the extender before use as described above
- a small portion of semen is mixed with a small volume of the extender and then evaluated
- providing that the extender has no deleterious effect, the remaining semen is diluted with the extender at 37 °C:
  (a) it is preferable to make a volume:volume dilution rather than to try and obtain a standard spermatozoal concentration
  (b) the optimal dilution rate appears to be 1:4 (semen:extender)
- following dilution the sample is re-evaluated, and then placed in insulated vials
- vials are slowly cooled to 5 °C and allowed to equilibrate for 2–4 hours
- diluted semen is then loaded into 0.5 ml straws in a cold room
- straws are frozen at standard freezing rates in liquid nitrogen vapour, before being plunged into liquid nitrogen
- straws are generally thawed rapidly (70 °C for 10 seconds) before being placed at 37 °C, or thawed at a medium rate by placing in a water bath at 37 °C
- uterine insemination is mandatory to achieve acceptable success rates when using frozen–thawed semen

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Insemination

- the accurate timing of insemination is essential when poor-quality semen is used, or when the use of cryopreserved semen is contemplated (this has short longevity in the female tract):
  (a) the ideal time is between 2 and 5 days after ovulation
  (b) for frozen semen, insemination should be performed on the basis of plasma (serum) progesterone (detection of the fertilization period) (see 11.2 and 11.3)
  (c) for chilled semen, insemination should be performed on the basis of plasma (serum) progesterone
  (d) for fresh semen, insemination may be performed on the basis of vaginal endoscopy and vaginal cytological assessment (detection of the fertile period)
- a minimum of 150×10^6 normal motile spermatozoa should be present in each inseminate
- fewer sperm may be used if uterine insemination is used
- natural mating results in vaginal contractions that propel the ejaculate cranially into the uterus; these contractions are generally absent when a bitch is artificially inseminated and therefore spermatozoal transport is reduced
- uterine insemination results in a higher success rate than does vaginal insemination:
  (a) uterine insemination is necessary for reasonable success with frozen semen
  (b) vaginal insemination may give reasonable success with fresh semen

Insemination techniques

Intravaginal insemination

- a rigid plastic catheter is required, about 30 cm long for a large bitch
- attach a plastic syringe; if this has a rubber plunger it is best to wash the syringe out first with distilled water as rubber is toxic to spermatozoa
- suck the inseminate into the catheter; if fresh semen is being used (small volume) it need not enter the syringe
- initially the catheter is introduced vertically through the vulval lips, but at the level of the ischium it is directed cranially; the vagina of the bitch is relatively long
- if the bitch will allow, the catheter can usually be palpated through the abdominal wall and the cervix is located as a hard structure cranial to it
- the bitch's hind limbs are raised off the ground and the semen expelled; it may then be necessary to inject air down the catheter to ensure that it is empty; homologous seminal plasma is a preferred flushing medium since this increases pregnancy rate [9]
- stimulating the bitch's vestibule with a finger is said to stimulate vaginal contractions which aid passage of semen into the uterus; vaginal stimulation also increases uterine contractions [9]

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- after 10 minutes, or if the bitch struggles, she is allowed to stand
- urination should be prevented for 10 minutes; reflux of a lot of fluid indicates a poor insemination
- the timing of insemination is discussed in 11.2; two inseminations should be made where possible, on alternate days

Intrauterine insemination

- it is very difficult to place a catheter through the bitch's cervix into the uterus because:
  (a) the vagina is long and narrow (up to 20 cm long)
  (b) the cervical opening is small, and placed at an angle to the vagina
- several methods have therefore been developed to allow uterine insemination:

Foley catheter technique

- an especially designed Foley catheter may be used to perform an effective uterine insemination:
  (a) the catheter has the bulb positioned at the catheter tip
  (b) a second catheter with a side exit port is inserted through the Foley catheter
- the catheter is inserted into the vagina up to the level of the cervix and the bulb is inflated; this forms a seal against the cranial vaginal wall
- the second catheter is advanced and the semen is deposited; semen can only run forwards through the cervix and does not drain back along the vagina
- the second catheter is withdrawn inside the Foley catheter, thereby closing the side exit ports
- both catheters are left in place for up to 15 minutes:
  (a) this ensures that a pool of semen is located next to the cervix
  (b) this may simulate the copulatory tie and result in vaginal contractions
- the principle of the technique is excellent, but there is often leakage of semen around and through the catheters

'Norwegian' catheter technique

- consists of an outer plastic catheter and inner metal catheter with rounded bulb-ended tip (Figure 28.1)
- the outer plastic catheter is inserted into the vagina to the level of the cervix
- the cervix is palpated transabdominally (Figure 28.2)
- the inner catheter is pushed forwards to the cervix and at the same time the cervix is re-orientated transabdominally; the aim is to produce a straight line from the vagina into the cervix so that the inner catheter can be directed through the cervical canal
- this technique works very well but requires training and considerable practice

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Figure 28.1 Norwegian catheter: a white coloured soft plastic outer catheter and inner metal catheter with round-bulbed end.



Figure 28.2 Using the 'Norwegian' catheter insemination technique: the cervix is located by transabdominal palpation and then re-aligned so that the inner rod can be pushed through the cervical os.

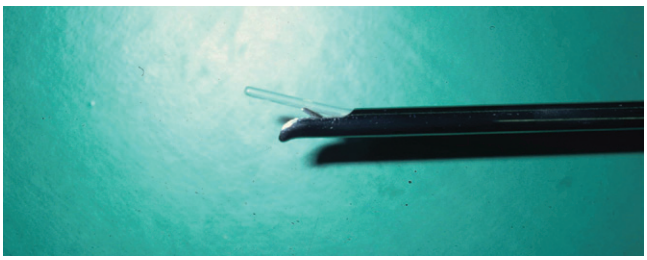


Figure 28.3 Endoscope with deflection device for direction of an inseminating catheter through the cervical os.

- it is impossible in large, obese or frightened animals with a tense abdomen
- this technique can be augmented by injection of radiographic contrast medium to ensure correct placement of the catheter through the cervix; the author has demonstrated that this has minimal effect on semen quality [10]

Endoscopic technique

- this method requires a rigid endoscope and inflation system, preferably with a mechanism for deflecting a catheter at the endoscope tip (Figure 28.3)

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- the endoscope is inserted into the vagina and advanced to the level of the cervix
- inflation of the vagina is useful for allowing the identification of the cervical os
- the os is positioned in the dorsal wall of the vagina
- the inseminating catheter is inserted through the endoscope and the deflection device is used to direct the catheter into the os
- the technique works very well, although a small number of bitches need to be sedated otherwise movement of the bitch makes placement of the catheter very difficult; reversible sedation with the alpha-2 adrenoreceptor agonist drugs such as medetomidine appears to be ideal
- the technique requires training and practice before catheterization can be achieved reliably

Surgical technique

- the ethics of performing surgery to achieve insemination have been questioned [2]; at least in the UK this procedure can only be performed if for valid reasons a non-surgical insemination cannot be achieved
- requires general anaesthesia
- a small caudal ventral midline laparotomy incision is made
- the uterine body is lifted into the incision
- a fine over-the-needle intravenous catheter is placed into the lumen of the uterine body
- padded bowel clamps are placed on the uterus distal to the catheter
- the semen is introduced slowly into the uterus and runs forwards into the uterine horns
- following removal of the catheter pressure is applied to the site to provide haemostasis; no sutures are required
- the incision is closed routinely
- the technique is simple and can be performed rapidly

NB: Our ethical appraisal of the use of AI in dogs was the first for reproductive technologies in this species and adds important information to the debate regarding these technologies [2].

Success with artificial insemination

- in the bitch there appears to be a substantial difference in spermatozoal longevity for fresh, chilled–rewarmed and frozen–thawed semen; the longest periods of time for sperm survival appear for fresh semen, with frozen–thawed semen having the poorest longevity (Figure 28.4) [8]
- fertility rates are related to spermatozoal damage during preservation and to spermatozoal longevity
- furthermore, we have shown that sperm abnormalities induced by cryopreservation result in decreased ability of sperm to attach to female reproductive tract epithelium and therefore contribute to the lower fertility [11] (see also 11.2)

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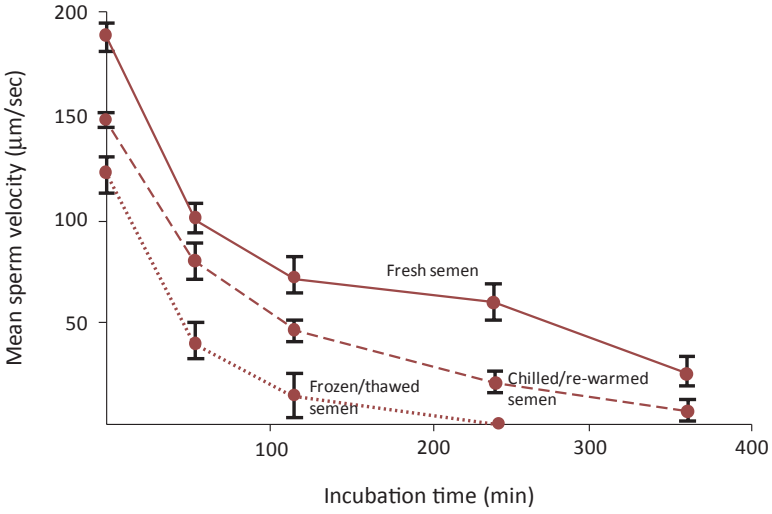


Figure 28.4 At time zero there are substantial differences in the quality of fresh, chilled–rewarmed and frozen–thawed semen; incubation in conditions similar to those found in the female reproductive tract results in the most rapid reduction in quality of frozen–thawed semen, and the least effect on fresh semen.

- extensive bitch management is required when frozen–thawed semen is used, since insemination has to be performed during the fertilization period
- it may be extremely difficult to preserve the semen from certain dogs; the reason for this is not known
- as a guide, 'world average' success rates for bitches inseminated with semen preserved and inseminated in different way is shown in Table 28.1

Table 28.1 Average (±variation) pregnancy rates for semen preserved and inseminated using different techniques. Litter sizes also vary.

	Vaginal insemination	Cervical insemination	Uterine insemination transcervical	Uterine insemination surgical
Fresh semen	80 ± 16	Not available	95 ± 4	95 ± 4
Chilled semen	47 ± 9	Not available	81 ± 19	Not available
Frozen–thawed semen	45 ± 24	60 ± 15	70 ± 11	95 ± 7

NB: In certain laboratories values may be higher than those in Table 28.1, especially when the semen collection and insemination are performed entirely in-house.

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28.2 ENHANCING FERTILITY IN MALES AND FEMALES WITH REPRODUCTIVE TRACT DISEASE [12]

Embryo transfer

- in some species where the female has uterine disease, embryos are harvested shortly after fertilization and then transferred to a recipient female who must be at a similar stage of the oestrous cycle to the donor
- transferring the embryos is often done surgically either via laparotomy or laparoscopically because this reduces the risk of introducing infection into the uterus that may occur if the transfer is done via the cervix and because the cervix is closed
- special culture media and specific temperature and humidity are required for optimal storage of the embryo between collection and transfer, and optimally the interval should be as short as possible
- experimental studies have demonstrated that embryos harvested from bitches at natural cycles can be preserved by freezing and can then be transferred non-surgically to recipient bitches
- at the moment collection methods have involved surgically removing the reproductive tracts and flushing them, but it is likely that in the future methods will be developed to allow transcervical collection of embryos; any such methods would be improved by regimes that increase the number of oocytes ovulated at a given oestrous cycle (termed 'superovluation')

In vitro fertilization

- in vitro fertilization can be a useful technique when only small number of oocytes are ovulated, or when there is disease of the reproductive tract but not ovaries, or where an animal dies but its ovaries can be quickly recovered
- the technique involves the collection of oocytes and fertilizing them in the laboratory when they are mature before they are transferred into a recipient female (immature oocytes can also be collected and matured in the laboratory before being fertilized)
- there are substantial difficulties with in vitro fertilization in bitches, notably the difficulties described for embryo transfer and the challenges of collection of oocytes without surgically removing the ovary; the latter has yet to be overcome in the bitch
- use of immature oocytes is fraught with difficulties because culture of canine oocytes is not particularly successful with maturation rates of approximately 25% [13,14,15]
- the author's laboratory has been preeminent in studying in vitro maturation of canine oocytes; data have added to knowledge of the basic biology as well as improving the potential for clinical application of the technique [16,17,18]
- only one successful pregnancy has been reported following the transfer of embryos created by in vitro fertilization in the author's clinic [19]; here

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pre-ovulatory oocytes were collected, so whilst this demonstrates the possible applicability of the technique it creates practical difficulties for it to be used in clinical practice

Intracytoplasmic sperm injection and related techniques

- the injection of a single sperm into a mature oocyte is described as intracytoplasmic sperm injection (ICSI)
- the technique is used in human fertility clinics where there are very few sperm available; potentially sperm harvested from the epididymis of recently dead dogs could be used
- whilst canine oocytes can be fertilized using ICSI, subsequent development of the embryos has not be shown and the technique as yet has no clinical applicability
- there are other similar techniques designed to help sperm cross the zona pellucida and fertilize oocytes:
  (a) zona drilling
  (b) partial zona dissection (PZD)
  (c) sub zonal injection (SUZI)
- as yet these have not been utilized in dogs

28.3 HARVESTING GONADAL TISSUE

- to date most of the reproductive biotechnology methods has used sperm collected at ejaculation, oocytes harvested after surgical removal of the ovaries, or embryo flushed after surgical removal of the tract
- essentially there are technical challenges to the techniques that are currently limiting clinical application
- however in some circumstances salvage procedures may be necessary to collect gonadal tissue from recently deceased animals

Salvage techniques in the bitch

- collection of ovaries immediately after death offers the potential for collection of oocytes which could potentially be frozen, or matured and fertilized before either being frozen or transferred; whilst these techniques are available in other species as of yet they have not been demonstrated to be effective in dogs

Salvage techniques in the dog

- collection of testes immediately after death allows collection of sperm from the tail of the epididymis; these sperm can be inseminated immediately or frozen and thawed

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- in the author's laboratory we conducted the principal study on cryopreservation of dog epididymal sperm [20]
- epididymal sperm are immature and success rates are lower than with ejaculated sperm but this technique has some clinical use in particularly valuable animals (most commonly it might be used for preservation of endangered canine species rather than the domestic dog)

28.4 CLONING

As currently practiced cloning is the technique of taking the nucleus of a mature cell and placing it into an oocyte that has had its genetic material removed (an enucleated oocyte).

- this is achieved by staining the genetic material of the oocyte so that it can be identified and sucked out of the oocyte, and then by injecting the nucleus of the adult cell (often a fibroblast because of their high developmental competence) into the peri-vitelline space of the oocyte; an electrical impulse is used to initiate the process of fusion and activation of the oocyte
- the new oocyte then contains the genetic material of the adult cell and it is placed into the uterus of a recipient animal to develop as a normal pregnancy
- in dogs successful clones have been produced using the nucleus of fibroblasts placed into enucleated oocytes
- technically the process is very difficult and expensive and only few clones are likely to be produced
- clones are only genetically identical to the adult cell which does not mean that they have the same phenotypic appearance, nor same temperament or characteristics of the donor animal

REFERENCES

1 Burgess, C.M., Bredl, J.C.S., Plummer, J.M. and England, G.C.W. (2001) Vital and ultrastructural changes of frozen-thawed dog spermatozoa. *Journal of Reproduction and Fertility* **57**(Supplement), 357–363.
2 England, G.C.W. and Millar, K.M. (2008) The ethics and role of artificial AI with fresh and frozen semen in dogs. *Reproduction in Domestic Animals* **43**, 165–171.
3 England, G.C.W. and Keane, M. (1996) The effect of X-radiation upon the quality and fertility of stallion semen. *Theriogenology* **46**, 173–180.
4 England, G.C.W. and Allen, W.E. (1992) Factors affecting the viability of canine spermatozoa I. Potential influences during processing for artificial insemination. *Theriogenology* **37**, 363–371.
5 England, G.C.W. and Allen, W.E. (1992) Factors affecting the viability of canine spermatozoa II. Effects of seminal plasma and blood. *Theriogenology* **37**, 373–381.
6 England, G.C.W. (1999) Semen quality in dogs and the influence of a short-interval second ejaculation. *Theriogenology* **52**, 981–986.

Vet-ebooks.com

7 England, G.C.W. and Ponzio, P. (1996) Comparison of the quality of frozen–thawed and cooled–rewarmed dog semen. *Theriogenology* **46**, 165–171.
8 England, G.C.W. (1993) The cryopreservation of dog semen – a review. *Journal of Reproduction and Fertility* **47**(Supplement), 243–255.
9 England, G.C.W., Moxon, R. and Freeman, S.L. (2012) Stimulation of mating-induced uterine contractions in the bitch and their modification and enhancement of fertility by prostatic fluid. *Reproduction in Domestic Animals* (in press).
10 England, G.C.W. and Verstegen, J.P. (1996) Radiographic contrast medium for uterine insemination in the bitch, and its effect upon the quality and fertility of fresh dog semen. *Theriogenology* **46**, 1233–1241.
11 Burgess, C.M., Clutterbuck, A. and England, G.C.W. (2011) The effect of cryopreservation on the capacitation status and epithelial cell attachment capability of dog spermatozoa. *The Veterinary Journal* In press.
12 Hewitt, D.A. and England, G.C.W. (2001) Manipulating canine fertility using in vitro culture techniques. *Journal of Reproduction and Fertility* **57**(Supplement), 111–125.
13 Hewitt, D.A. and England, G.C.W. (1997) Effect of preovulatory endocrine events upon maturation of oocytes of domestic bitches. *Journal of Reproduction and Fertility* **51**(Supplement), 83–91.
14 Hewitt, D.A. and England, G.C.W. (1998) The effect of oocyte size and bitch age upon oocyte nuclear maturation in vitro. *Theriogenology* **49**, 957–966.
15 Hewitt, D.A., Watson, P.F. and England, G.C.W. (1998) Nuclear staining and culture requirements for in vitro maturation of domestic bitch oocytes. *Theriogenology* **49**, 1083–1101.
16 Hewitt, D.A. and England, G.C.W. (1999) Culture conditions required to induce capacitation and the acrosome reaction of canine sperm in vitro. *Veterinary Record* **144**, 22–23.
17 Hewitt, D.A. and England, G.C.W. (1999) Influence of gonadotrophin supplementation upon in vitro maturation of domestic bitch oocytes. *Veterinary Record* **144**, 237–239.
18 Hewitt, D.A. and England, G.C.W. (1999) Synthetic oviductal fluid and oviductal cell co-culture for canine oocyte maturation in vitro. *Animal Reproduction Science* **55**, 63–75.
19 England, G.C.W., Verstegen, J.P. and Hewitt, D.A. (2001) Pregnancy following in vitro fertilisation of canine oocytes. *Veterinary Record* **148**, 20–22.
20 Hewitt, D.A., Leahy, R., Sheldon, I.M. and England, G.C.W. (2001) Cryopreservation of epididymal dog spermatozoa. *Animal Reproduction Science* **67**, 101–111.

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Index

abortion 225–229
  bacterial causes 226
  habitual, progestagens and 291
  induction 276–277
  partial 229
  prevention of 229, 291
  of unknown aetiology 229
  viral causes 226–228
abscess
  post-renal 281
  prostatic 199
accessory nipples 173
acromegaly 288
acrosome abnormalities 96
activated sleep pattern 149
activins 76
acute phase proteins 132
acyline 303
afterbirth *see* placenta
agalactia 243–244, 312
aggression
  following orchidectomy 285
  reducing, using progestagens 291
aglepristone 274, 276
Agpar score 246–247
alkaline phosphatase 97, 195
allantochorion 123, 144
allantois 123, 134
alopecia
  and progestagen 288
  and testicular tumours 194
  bilateral non-pruritic 293
alpha-adrenergic drugs 176, 303
alpha-reductase inhibitors 198, 202, 313–314
amnion 134
anabolic steroids 218
anaemia
  and oestrogens 293
  in pregnancy 125
anal adenoma 292, 295
anasarca 230
ancylostoma 7
androgen binding protein 75
androgens 39, 75, 296–298
  adverse effects 219, 296
  and prostatic disease 197
  cause of enlargement of clitoris 159, 296
  cause of intersex 207
  contraception 277, 298
  control of oestrus 269, 271, 296–297
  treatment of poor libido 218, 297
  treatment of poor semen quality 297–298
  treatment of pseudopregnancy 216, 297
androstenedione during metoestrus 39
anoestrus 31, 37
  duration 31
  endoscopic examination 51, 117
  ovaries in 25, 55
  primary 209, 257, 299
  secondary (prolonged) 212
  uterus in 23
  vagina in 43
  vaginal cytology 43
anorchia 184
anorexia in pseudopregnancy 214
anovulation 210
anti-androgens 197, 202, 291, 313–314
  adverse-effects 313
  effects 313
  treatment of prostatic disease 313–314
antibiotics
  in managing abortion 229
  in mastitis 241
  in perivulval dermatitis 157
  in prostatic disease 199
  in pyometra 170
anti-emetics 308
antisocial behaviour 291
anuclear cell index 48, 115

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arginine esterase 198
artificial insemination 315–324
  advantages 315–316
  chilled semen 317–319
  disadvantages 316
  fresh semen 317–319
  frozen semen 317–319
  lifespan of sperm 317
  regulations 316
  success 323–324
  technique 320–323
  timing of 320
artificial vagina 87
atresia, congential vulval, correction of 157
atrophic vaginitis 164
attractiveness to other male dogs 221
azoospermia 97, 188, 219

bacteria
  and abortion 236
  anaerobic 59
  in anoestrus 59
  in male reproductive tract 59, 103, 182
  in mastitis 244
  in metoestrus 59
  in oestrus 58–60
  in pro-oestrus 58–60
  overgrowth in neonates 251
  prepuce, interpretation 103
  prostatitis 198–199
  pyometra 59, 166–167
  uterus 59
  vaginal, interpretation 58, 59, 78
balanitis 180
balanoposthitis 182
basenji, anoestrus in 31, 212
benign prostatic hyperplasia 196–198, 291, 295
blastocoele 123
blastocyst 123
blood in ejaculate 88, 95, 198
body pregnancy 225
body condition score 8
bone marrow suppression 293
breach birth 145
breeding soundness examination 17
  female 41
  male 83
bromocriptine 307
Brucella aborutus 226
Brucella canis 78, 104, 187, 226, 262
Bulbus glandis 64, 87
Buserelin 60, 300

cabergoline 32, 215, 242, 307
caesarean operation 141
calcium
  during pregnancy 242
  supplementation 239
canine adenovirus 228
canine distemper virus and abortion 227
canine herpes virus 140, 159, 224, 226–227, 262, 317
canine parvovirus 228
cannibalism 243
care of the bitch after parturition 146
castration *see* orchidectomy
cerebellar degeneration 227
certificate of mating 82
cervical mucus 49, 118
  and oestrogens 49
  ferning, patterns 49–50, 116
cervix (cervical os) 21
  anatomical abnormalities 164
  bacterial flora 59
  dilation at parturition 144
chromosomal sex, abnormalities 205–206
chromosomes 12, 205
cleavage 122
clitoris
  englargement of 159, 207
  hypertrophy and androgens 296
cloning 327
cloprostenol 169, 304
coat changes
  after ovariohysterectomy 282
  following progestogens 288
coitus *see* mating
colostrum 151–152
colposuspension 176
conception
  failure 223, 255, 259
  prevention of 274–276, 294, 304
conceptus, development of 123, 133
contraception, hormonal, in the bitch 267–278
contraception, hormonal, in the dog 277–278, 292, 298
copulation failure 255, 259
cornification of vaginal cells 48, 115
corpora lutea 25, 31, 38
corticosteroids
  and termination of pregnancy 277
  at parturition 137
C-reactive protein 132
cremaster muscle 70
cyproterone acetone 313
cryptorchidism 76, 184–186, 285, 300
crystallization of cervico-vaginal mucus 49, 116
cystic endometrial hyperplasia 165, 223, 229
cystitis 199

Vet-ebooks.com

cysts
  ovarian 212
  prostatic 200
cytological examination of pathology 60
cytoplasmic droplets 70, 220

dartos muscle 70
dehydration 249–250
delayed puberty *see* anoestrus primary
delmadinone acetate 291
dermatitis, vulval and perivulval 157
deslorelin 35, 257, 269, 300
diabetes 125, 188, 231, 289
diarrhoea in neonates 253
digital examination 50
diethylstilboestrol
  and oestrus-induction 294
  and prostatic hyperplasia 197, 295
  to prevent conception 275, 294
Diff Quik 47
Dihydroestosterone 75, 296
Dinoprost 304
dioestrus
  androstenedione in 39
  cystic endometrial hyperplasia 165
  cytology, vaginal 43, 45
  digital examination 50
  duration 31
  endoscopic examination 50–52
  ovaries in 25
  progesterone in 38
  uterus in 23
  vagina in 43
  vulval changes 113
Dirofilaria 7
Distal cytoplasmic droplets 70, 220
DNA 12
dog pox 181
dorsal median fold 50
ductus deferens 66, 70
dystocia
  and oxytoxin 232, 239, 311
  definition 232–233
  investigation of 234–239

eclampsia
  in late pregnancy 141, 239, 242
  subclinical 242
  suppression of lactation 242, 309
ectoparasites 6
ectopic testes 76, 186
eczema of scrotum 188, 203
ejaculate fractions 68, 72, 85, 88, 196
ejaculation
  failure 189, 195, 259, 262
  retrograde 220
electrical resistance of vagina 118
embryo 123, 133
embryonic death, early 224–225
endometrial glands 23, 165
endometritis 166, 229
endoparasites 6
endoscopic examination 50–52, 116–118, 163
  changes through oestrus cycle 51
  interpretation 51
  optimal time to breed 51, 116–118
endoscopic insemination 322–323
endotheliochorial placenta 124
enzyme linked immunosorbent assay (ELISA) 56, 114, 141, 235
embryo transfer 325
eosin 90, 191
epididymis 66, 70
  accelerated sperm passage through 220
  anatomy 66
  anatomical abnormalities 195
  transit time 70
  ultrasound examination 99
epididymitis 195
episiotomy 162
equine chorionic gonadotrophin (eCG) 298
erection 71, 178, 259
ergot preparations 310–312
  effects 310–311
  treatment of fetal retention 311–312
  treatment of placental retention 311–312
  treatment of post-partum haemorrhage 312
  treatment of uterine inertia 311
erythrocytes, in semen sample 88, 95, 198
erythropoiesis 168
Escherichia coli
  and abortion 226
  in pyometra 166
  in vagina 58
estimated breeding value 11
ethinyl oestradiol 216
eutocia, definition 232

fading pups 252
failure to conceive 255, 258
failure to copulate 259
Fallopian tubes *see* uterine tubes
false pregnancy *see* pseudopregnancy
feminizing syndrome 194–195, 221
Fenbendazole 140
fertile period 30, 111
fertility
  definition 254
  expectations of 254, 263

Vet-ebooks.com

fertilization 110
  failure 223
  period 30, 110
fetal fluid, loss of 230
fetal membranes 123
fetal membranes, retained 241, 311–312
fetus
  abnormalities 230
  number, estimation of 131, 136
  orientation 145
fibrinogen 132
fibroleiomata 60, 162
fimbrae 26
finasteride 198, 202, 313
fluid therapy, neonatal 250–251
flutamide 202, 313
follicle
  granulosa cells 24
  of oestrus 29
  ovulation 37, 55
  primordial 24
follicle stimulation hormone (FSH) 36, 74
  puberty 10
  in hypogonadism 278
  in male dogs 103
follicular cysts 172
food in pregnancy 141
foreplay in mating 78–79
formestane 202, 313
fractions of the ejaculate 68, 72, 85, 88
frenulum, persistence 180

galactopoiesis 126
galactostasis 245, 309
gelding *see* orchidectomy
genetic finger printing
genetics
  genetic disease 11
  genomic breeding value 11
  genotype 11
  dominants 12, 16
  epistasis 14
  heterozygous 12, 17
  homozygous 12, 17
  inbreeding 16
  line breeding 16
  recessives 12, 16
  outcrossing 16
  polygenetic 16
  polymorphism 13
  sex determination 14
  sex linkage 15
germ cell tumours 60
gestation period *see* pregnancy length
glucagon 125
glucocoricoid therapy 221
glucose in vaginal fluid 118
gonadal sex, abnormalities 205
gonadotophin releasing hormone (GnRH) 35, 74, 300–303
  adverse effects 301
  agonists and antagonists 35, 300, 303
  analogues 35, 300
  contraception 277, 303
  control of oestrus 269, 302
  effects 300–301
  identification of ovarian tissue 301
  immunization 271
  induction of oestrus 257, 302
  hastening ovulation 301
  prostatic disease 198, 303
  puberty 10
  stimulation test 60, 105
  treatment of incontinence 176
  unwanted mating 302
  urinary incontinence 302
gonadotrophins 35, 298–300
  adverse effects 298–299*
  diagnosis of testicular tissue 185, 300
  effect 298
  hastening ovulation 299
  identification of ovarian tissue 300
  induction of oestrus 259, 299
gonads, harvesting 326–327
gonads, intra-abdominal, removal 186
granulosa cells 24
granulosa cell tumours 173
growth curves 8, 151
growth plate closure 7
gynaecomastia 194

haemaspermia 197
haematuria 187, 197
haemocytometer 89
haemolytic streptococci 58–59, 163
haemophilia 15
haemorrhage
  and ovariohysterectomy 281
  following orchidectomy 285
  penile 88
  post-partum 242
heartworm 7
hermaphrodite 205
hernia, inguinal (scrotal) 203
hookworm 7
hormone assay 55
human chorionic gonadotrophin (hCG) 298
  adverse effects 298
  in mating 299
  in follicular cysts 172
  in oestrus-induction 299

Vet-ebooks.com

in ovulation induction 210, 212
in prolonged pro-oestrus 210
stimulation test 61, 76, 105, 185
hydrocele 230
hydrops, fetal 230
hymen 20, 160
hymenal remnant 50
hyperpigmentation, epidermal and oestrogens 293
hyperplasia
benign in prostate gland 196–198, 291–293, 295, 313–314
cystic endometrial 165, 223, 229
of vaginal mucosa 161
lymphoid 159, 181
hypocalcaemia 239, 242
hypoglycaemia 231
hypoglycaemia, neonatal 151, 248
hypogonadism 218, 300
hypo-osmotic swelling test 93–94
hypoplasia
of tests 186
vulval 157
hypospadias 180
hypothalamic-gonadal axis 36, 74
hypothermia 150–151, 248
hypothyroidism 209, 216
hysterotomy *see* caesarean operation

implantation 124
impotence 221
inability to mate 258
inbreeding 16
inbreeding coefficient 17
incontinence
after ovariohysterectomy 176, 282–283, 295
in intersexuality 207
in the bitch 217
infertility
approaches to 254–264
definition 254
in kennels 262–263
in the bitch 255–259
in the dog 259–262
lack of knowledge about 263–264
infrequent oestrus 255
inguinal canal 67
inhibin 76
insemination *see* artificial insemination
insulin 125
interoestrous period
long 212
short 211–212
intersexuality 205
aetiology 205–207
clinical signs 207
external physical signs 208
hypospadias in 208
internal reproductive organs 208
sex determination 14
treatment 208
interstitial cell stimulating hormone (ICSH) *see* luteinizing hormone
interstitial cell tumour 194
interstitial cells 74, 219
intra-cytoplasmic sperm injection (ICSI) 326
intra-osseus fluids 250
intra-peritoneal fluids 250
intra-uterine insemination 321–323
intra-vaginal insemination 320–321
intromission in mating 79, 81
in vitro fertilization 325–326

juvenile vaginitis 163, 280
juvenile vulva 157

karyotyping 62, 107, 206, 209
keratinization of vaginal cells 43
ketoconazole 261
King Charles spaniels, urination problems in 197

labour *see* parturination
lacatation
in pregnancy 126
in pseudopregnancy 38–39, 213–214
prolactin in 38–39, 214
laparoscopy and laparotomy 62, 165
laparoscopic ovariectomy 283
leiomyoma 60, 162
Leishman's stain 47
Leydig cells 74, 219
Libido 80, 218, 300
Line breeding 16
Luprostriol 304
Luteal cyst 172, 212
luteal phase, short 210, 229
luteotrophic action 58
luteinizing hormone (LH) 36, 74
and abnormal spermatogenesis
in hypogonadism 218
in male dogs 102
measurement 114
peak 37, 56, 58, 110
puberty 10, 210
role 58
lumbosacral disease 81
lymphoid hyperplasia 159, 181

mammary glands 26
asymmetry 173
blood supply 27

Vet-ebooks.com

mammary glands (*cont'd*)
fibroadenomatous hyplerplasia 174
lymphatic drainage 27, 175
mastitis 244–245
number 26
palpation 42
pregnancy 125
tumours 174–176, 203, 288
marginal haematoma 124
mastitis and pseudo pregnancy 32
mating 72
difficulties 255
factors preventing normal 80, 255, 258
normal behaviour 79, 103
planning 77
timing 77, 109
and fertilization failure 223
optimal, determination 109–119
mediastinum testis 99
medroxyprogesterone acetate 288–290
megestrol acetate 289
meiosis 69
mésalliance *see* unwanted mating
mesentery 26
mesometrium 26
mesosalpinx 26
mesovarium 26
mesterolone 189, 192, 298
metaplasia of protate gland 199, 293
methyl-p-hydroxybenzoate 85
methylene blue 47
metoclopramide 308
metostrus *see* dioestrus
metorrhagie 171
metritis, post-partum 241
metrocoele 230
mibolerone 297
milk
at parturition 144
composition in relation to parturition 137, 236
excretion 126
letdown 243, 312
production 42
misalliance *see* unwanted mating
modes on inheritance 12–15
monorchidism 184
morula 122
mosaic 206
mounting and thrusting behaviour in bitches 216
mucosa, vaginal *see* vaginoscopy
Mullerian ducts 160
myoepithelial cells 27, 126, 310

neonate
artificial rearing 152
behavioural development 3, 148–149
causes of death 251–252
clinical examination 246
critical period 4
deworming 6, 152
dietary requirements 5
growth 7, 9, 151
healthcare 150–154, 247
neonatal period 4, 149
physiology/husbandry 148
resuscitation 150
socialisation period 3–5
transitional period 4, 149
treatment 247–251
neoplasia
cervix 164
ovarian 173
penile 183
prostate 200–202
transmissible venereal tumour 60, 158, 183, 317
testicular 193–195
uterine 171
vaginal 162
neutering *see* orchidectomy
neutropjilia 169
neutrophils, in semen sample 191–192
nidation 124
nigrosin-eosin 90, 191
nipples
abnormalities 173–174
Norwegian catheter 321
nucleotide 12
nutrition, neonatal 5

obesity
following orchidectomy 285
following ovariohysterectomy 282
obestrical manipulation 239–240
oestradiol benzoate
adverse effects 275–276
and oestrus induction 257, 294
and prostatic hyperplasia 197, 295
to prevent conception 275
oestrogen responsive dermatosis 217
oestrogens 293–295
adverse effects 219, 293
and oestrus induction 258, 294
and pyometra 293
and Sertoli cell tumours 193, 207, 261
and vaginal hyperplasia 161
at parturition 121
effect on mammary gland 27
effect on perineum and vulva 113
effects 37, 293
in metoestrus 58
in pregnancy 58, 121
in proestrus 58

Vet-ebooks.com

treatment of anal adenomata 295
treatment of juvenile vaginitis 294
termination of pregnancy 275
treatment of prostatic disease 197, 202
treatment of pseudopregnancy 216
treatment of unwanted mating 294
treatment of urinary incontinence 176, 295
oestrous cycle
irregular 209–213, 285
terminology 28–31
vulval changes 113
oestrus 29
advantage of prevention and suppression 267
behaviour 29, 112
cervical canal in 22
digital examination 50
disadvantage of prevention and suppression 267
endoscopic examination 50–52, 116–118
future methods of prevention 271
induction 257–258, 294, 299
and pseudopregnancy 294
and hyperplasia of vaginal mucosa 161
induction
and GnRH 257
and gonadotrophins, side-effects 257
and oestrogens 258, 294
prolactin inhibitors 257
ovaries in 25
persistent 210
post-spay 281
prevention 267–271
relation to ovulation 29–30, 78, 112
suppression 267–271
unwillingness to mate during 159–160, 213, 255, 259
uterus in 23
vagina in 21, 42–45, 50–52, 117–118
vaginal cytology 43, 45
vulval changes 113
oocyte 30, 122, 325
optimal time to breed 48, 112–118
orchidectomy 284
indications 284
operative and post-operative complications 285
timings 284
orchitis, traumatic 186
orchitis, non-traumatic 187
organogenesis 123
orogastric tube 153
osaterone 291, 313
os clitoridis, enlarged 159, 207
os penis 64, 68, 72
frature of 181
in intromission 79
outcrossing 16, 17
ovarian artery 26
ovarian bursa 24, 26
ovarian cysts 172
ovarian imbalance 216
ovarian remnant 60–61, 281–282
ovarian tumours 173
ovarian vein 26
ovariectomy 283
ovariohysterectomy 279
and pseudopregnancy 282
and vagina prolapse 162
for pyometra 169
for removing pregnancy 274
indications 279
operative and postoperative complications 281–283
timings 279–281
ovary 24
cysts 172
histology 24–25
identification 300
salvage 326
tumours 173
ultrasound examination 55
overdue bitch 142, 233–236
overuse of stud dog 190, 220
ovotestes 207
ovulation 29, 30, 78
failure 221
hastening 299, 301
in oestrus 30, 110–112
in pro-oestrus 29–30
luteinizing hormone in 37, 55, 58, 110
progesterone in 38, 56, 110, 14
time 30
and mating 109, 212
and progesterone 38, 56, 110, 114
ultrasound detection of 55
unpredictability 30, 213
ovum 122
oxytocin 310–312
at parturition 137, 232
effects 126, 310–311
induction of parturition 146
promoting milk let-down 126, 243, 312
treatment of fetal retention 239, 311, 312
treatment of placental retention 242, 311–312
treatment of post-partum haemorrhage 242, 312
treatment of post-partum metritis 242, 312
treatment of sub-involution of placental sites 242, 312
treatment of uterine inertia 239, 311

palpation and diagnosis of prostatic disease 197, 198, 201

Vet-ebooks.com

pampiniform plexus 66, 70, 187
paraphimosis 178
paraprostatic cyst 200
pars longa glandis, of penis 64
partial abortion 229
parturition
  cervical canal in 144, 236
  environment for 138
  first stage 143
  haemorrhage 242, 312
  induction of 146, 310
  ineffectual straining 237
  nervous inhibition of 237
  normal 146
  obstetrical manipulation 239–240
  onset and duration 137
  'overdue' bitch 233–237
  preparation of bitch 140–142
  presentation, position and posture 145, 237–238
  pre-partum hypothermia 120, 236
  progesterone and 136, 235
  prolactin and 137
  relaxin and 122, 137
  second stage 144
  third stage 146
parvovirus 193
penile haemorrhage 180, 197
penile tumours 183
penis 64
  anatomical abnormalities 180
  bacterial flora 103–104
  examination 84
  haemorrhage 180
  hypoplasia 180
  hypospadius 180
  neoplasia 183
  persistent frenulum 180
  persistent protrusion 179
  posthitis 181
perineum, examination of 42
perivulval dermatitis 187
phantom pregnancy *see* pseudopregnancy
phenotypic sex, abnormalities of 205
phenylpropanolamine 303
pheromone
  and erection 71
  and synchrony of oestrus 31
  production and oestrogens 37
phenotype 11
phimosis 178
physical development 7, 9
placenta
  chorioallantoic 123
  development 124
  retention, treatment of 241
  separation during birth 236–237
  sites, subinvolution 242
polymorphonuclear leucocytes
  in semen sample 191–192
  in vaginal smear 48, 115
polyps, vaginal 162
post-spay pseudopregnancy 214, 282
posthitis 181
pregnancy
  abdominal enlargement in 125
  acute phase proteins 132
  anaemia in 125
  appetite in 125
  behavioural changes 124, 129
  blood changes 128
  development of conceptus 122
  development of placenta 124
  diagnosis
    acute phase proteins 132
    blood tests 131–132
    detection of fetal heart 130
    palpation 129–130
    radiography 130
    real-time (B-mode) ultrasonography 132–136
    return to oestrus 129
  endocrine changes in blood 121–122
  failure 223–229, 255
  haematology 125
  length 38, 109, 120, 128, 142, 233
  mammary development 125, 129
  milk production in 125
  mucoid vulval discharge in 126
  termination 271–277, 294, 304, 306, 310
  uterine body 225
pregnant mare serum gonadotrophin (PMSG) 298
prepubertal vaginitis 163–164, 280, 294
prepuberty 28
  ovaries in 24
  uterus in 22
  vaginal epithelium, histological changes 42
prepuce 64, 84
  anatomical abnormalities 178–180
  balanitis 180
  opening, enlarging 179
  pendulous 194–195
  short 179
preputial discharge 182
prevention of implantation 274–276
pro-oestrus 29
  cervical canal in 22
  digital examinations 50
  first, prolonged 210
  oestrogens in 29, 37

Vet-ebooks.com

ovaries in 25
prolonged, progestagens in 211
relation to ovulation 29–30
uterus in 23
vagina in 20–21
vaginal cytology 42, 43
vulval change 113
progestagens 287–292
adverse effects 207, 219, 287–289
association with pyometra 288
control of oestrus 210, 268–270, 289–290
treatment of antisocial behaviour 291
treatment of circum and adenomata 292
treatment of follicular cysts 172
treatment of habitual abortion 291
treatment of prostatic disease 197, 202, 291–292
treatment of pseudopregnancy 215, 290
progesterone
antagonists 306–307
adverse effects 306
effects 306
induction of parturition 146, 307
pregnancy termination 274, 276, 306
treatment of fibroadenomatous hyperplasia 174
treatments of pyometra 166, 170, 307
and abortion 211
and ovulation time 56, 114
and prolactin release 39, 213
and pseudopregnancy
assay/measurement 38, 235
concentrations 57, 114
effects
on mammary gland 27
on uterus 23
in metoestrus 38, 55
in milk 236
in parturition 121, 136, 235
in pregnancy 57, 121, 141
measurement in unwanted mating 273
pre-ovulatory rise 38, 56, 110, 114
receptors 167
prolactin 38
behavioural effects 39
inhibitors 307–310
adverse effects 308–309
effects 307
induction of oestrus 257, 310
in mammary surgery 176
termination of pregnancy 277, 310
treatment of fibroadenomatous hyperplasia 174
treatment of galactostasis 309
treatment of pseudopregnancy 215, 309
treatment of pyometra 169, 309
at parturition 122
in lactation 122
in pregnancy 38, 122, 136
in pseudopregnancy 32, 38
release, progesterone and 38
prolapse of hyperplastic vagina 161
proligestone 288
prolonged anoestrus 212
prolonged pro-oestrus 210
promoting milk let-down 126, 243, 312
prostaglandins 39, 304–305
adverse effects 304
and prolactin inhibitors 309–310
at parturition 39
effects 304
induction of oestrus 305
termination of luteal phase 40
termination of pregnancy 277, 304
treatment of post-partum metritis 305
treatment of pyometra 169–170, 305
prostate gland 68, 71
hyperplasia 196–198, 291–292, 295, 313–314
side-effects of oestrogens and 199
ultrasound examination 100–102
prostatic abscess 199
prostatic biopsy 104
prostatic cysts 200
prostatic disease
and abnormal sperm 221
and mating behaviour 81
diagnosis 197–202
effect on sperm 202
metaplasia 199, 293
treatment 202, 291–292, 295, 303, 313–314
prostatic fluid 71, 72
prostatic massage 105
prostatic tumours 200–202, 292, 295, 314
prostatitis 71, 198–199, 292
proximal cytoplasmic drop (bead) 70, 220
pseudocyeis *see* pseudopregnancy
pseudohermaphrodite 205
Pseudomonas spp.
and abortion 229
in vagina 59
pseudopregnancy 27, 31, 213
and bromocriptine and cabergoline 307–310
and ovariohysterectomy 214
clinical signs 214
iatrogenic 32, 309
in metoestrus 31
irregular oestrous cycles and 33
milk production in 214
overt and covert 32
post-spay 214, 282

Vet-ebooks.com

pseudopregnancy (*cont'd*)
previous litter and 33
progestagens in 290
progesterone and 32, 38
prolactin in 32, 214, 309
pyometra and 33
treatment 215, 290, 294, 309
psychological inability to mate 255
puberty
breeding at first oestrus 18
delayed 209, 257, 299
normal 9–11
sperm production 69
puppy *see* neonate
puerperal tetany141, 239, 242
pyometra
aetiology 166–138
and intersexuality 207
and irregular oestrous cycles 167
and litter number 168
and oestrogen 168
and progestagen 288
and progesterone antagonists 307
and prolactin inhibitors 309
and prostaglandins 305
and pseudopregnancy 33, 167
as indication for ovariohysterectomy 169
clinical signs 168
'closed' or 'open' 168
diagnosis 168–169
discharge 168
following ovariohysterectomy 283
stump 283
treatment 169–171, 305, 309–310

radiography 130, 235
real-time (B-mode) ultrasonography 52–53
and pregnancy diagnosis 132–136
examination of the bitch at term 234–235
identification of pregnancy loss 224–225
relaxin 122, 131
residual bodies 69
resorption 123, 224–225
respiratory disease, in neonates 253
retained fetal membranes 241, 311–312
retrograde ejaculation 220
roaming
use of oestrogens and progestagens to control 291, 295

Salmonella and abortion 226
salpingitis 223
scrotum
examination 84
eczema 219
hernia 203
swelling following orchidectomy 285
trauma 203
semen
chilled 317–319
collection and diagnosis of prostatic disease 84–88
prerequisities 84
procedure 83, 86
evaluation 88–98
reasons for 84
freezing 319
fresh 318
normal semen quality 190–192
predicting fertility 190–192, 223
processing 317–319
quality 96–97
and infertility 190–192, 261–262
effect of drugs on 288, 293
normal 190
relation to testicular size 99–100
thawing 319
seminiferous tubules 65, 69, 70
seminomata 194
Sertoli cells 69, 76
Sertoli cell tumours 193–194, 207, 261
sex reversal 207
sheath *see* prepuce
Shorr's method 46
silent heats 213
sperm
abnormalities 92–93, 288, 293
concentration, measurement 89
function 192
life span 111, 323–324
morphology 90–93, 95, 190, 261
motility 90, 93, 191
impaired 262
numbers 89, 190, 261
other cells 95, 191
penetration assays 94
predicting fertility 96–97
survival in the bitch 73, 77, 110
total output 65, 86, 190
transport 70, 73
vital staining 90, 191
spermatic artery 66
spermatic cord 66
spermatic vein 66
spermatids 69
spermatocoele 193
spermatocytes 69
spermatogenesis 65, 69
abnormal 189, 218–219
inhibition 71
temperature control 70

Vet-ebooks.com

spermatogenic arrest 189, 219
squamour prostatic metaplasia 199–200, 293
staging of mammary tumours 175
staphylococci in vagina 58
sterility, definition 254
steroidogenesis 75–76
stilboestrol and oestrus-induction 294
stilboestrol *see also* diethylstilboestrol
stillbirths 227
streptococci
  β-haemolytic 59, 226
  in pyometra 166
  in the male reproductive tract 103
  in vagina 58
stump pyometra 283
subfertility, definition 254
subinvolution of placental sites 242, 312
surgical insemination 325
suspensory ligament 26
sympathomimetics 220

tapeworm 6
temperature
  fall during parturition
termination of pregnancy 276–277
testes
  anatomy 65
  anorchidism 184
  atrophy 188–189
  biopsy 104
  cooling of 70
  cryptorchid 184
  cysts 193
  degeneration 188, 203, 219
  diagnosis of testicular tissue 105, 185, 300
  ectopic 219
  fine needle aspiration 104
  hydrocele 193
  hypoplasia 186
  measurement of testicular volume 99
  monorchidism 184
  normal descent 10, 65
  orchitis 186–187
  palpation 84
  salvage 326
  torsion 187
  tumours 186, 193–195, 219
  ultrasound examination 98–100, 183
testicular artery 66
testicular biopsy 104
testicular torsion 187–188
testicular tumours 186, 193–195, 219
testicular vein 66
testosterone 75
  and cryptorchidism 298
  and impotence 297
  and prostatic disease 197
  in mating 81
  normal values in dogs 102
thrombocytopaenia 293
thyroid deficiency 209, 216
tie in mating 79
tie, failure to 81
tocodynomometry 143
total sperm output 65, 89, 190
Toxocara canis 6
Toxoplasma gondii 228
transabdominal palpation 129–130
transducers, ultrasound 53
transmissible venereal tumour 60, 158, 183, 317
trisomy 209
tumours *see* neoplasia

ultrasonography 52, 98
  benign prostatic hyperplasia 197–198
  colour flow and contrast-enhanced 102, 201
  cystic endometrial hyperplasia 165
  embryonic resorption 224–225
  overdue bitch 234
  principles 52–53
  prostatic cysts 200
  pyometra 169
ultrasound terminology 52, 53
ultrasound transducer 52, 53, 98
umbilical hernia 145
unpredictable ovulation time 213
unwanted mating 271
  detection of sperm in 273
  progesterone assay in 272–273
  treatment 274–277, 294
  vaginal cytology in 272
unwillingness to mate during oestrus 217
urethra 20, 58
  prolapse 196
  sphincter mechanism incompetence 176, 282
  sub-mucosal collagen 176
  tumours 177
  urethropexy 176
  washings 105, 198
urination
  in pro-oestrus 29
  marking in bitches 217
  on chest and front legs 179
uterine artery 26
uterine contractions 143, 310
uterine ineria 141, 232–233
  primary 232, 236
  secondary 233, 239
  treatment 239, 311
uterine insemination 321
uterine prolapse 243

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uterine tubes 24, 26, 122, 172
uterine vein 26
uteroverdin 124
uterus
  anatomy 22
  aplasia 164–165
  bacterial flora 59
  cyclical changes 23
  cystic endometrial hyperplasia 165, 223, 229, 293
  prolapsed 230, 243
  rupture 230, 240
  torsion 241
  tumours 171
  ultrasound examination 54

vaccination 6, 78, 140, 227
vagina
  anatomy 20
  anatomical abnormalities 42
  anatomical changes 20
  artificial 87
  bacterial flora 58–60, 166
  constriction 160
  contrast study 21
  dorsovental band 160
  exfoliative cytology
  interpretation of bacteriology 59–60
  stenosis 160
vaginal cysts 164
vaginal cytology 31, 42, 44, 114–116, 163
  and pregnancy diagnosis 45, 49
  and unwanted mating 273
  collection 46
  interpretation 48
  quality assurance 42
  relation to ovulation 115, 213
  staining 46
  unwanted mating 272–273
vaginal discharge
  causes of 162–164
  collection of samples 46
  crystallization (ferning) patterns in 49–50, 116
  haemorrhagic
    at term 236
    following ovariohysterectomy 281
  interpretation of mucus crystallization 49–50, 116
vaginal endoscopy 50–52, 116–118, 163
vaginal epithelium histological changes 42
vaginal hyperplasia 161
  and infertility 161
  removal 162
vaginal insemination 320
vaginal prolapse *see* vaginal hyperplasia
vaginal smears *see* vagina cytology
vaginal stump haemorrhage 281
vaginal tumours 162
vaginitis 48, 162
  and androgens 296
  atrophic 164
  diagnosis 48
  oestrogen and 294
  prepubetal (juvenile) 163–164, 280, 294
vas deferens
  anatomical abnormalities 195
vasectomy 286
vestibule
  anatomy 19
  anatomical abnormalities 159
  bacterial flora 58–59
vestibule-vaginal junction 20
vital staining of sperm 90, 190
vulva
  anatomy 19
  anatomical abnormalities 157
  juvenile 157
  normal discharge 20
  swelling and oestrogens 29
vulval discharge 162–164
  and subinvolution of placental sites 242
  in pro-oestrus and oestrus 29
  mucoid 126
  post-partum 171
vulval hypoplasia 157
vulval softening 113
vulval stenosis 157

Weaning 153–154
whelping *see* parturition

yolk sac 123, 133

zona pellucida 123
zonary placenta 123, 124, 134
zygote 122

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